THE EFFECT OF BIOTIC AND ABIOTIC FACTORS WITHIN THE SOIL ENVIRONMENT ON THE MORTALITY OF *IXODES SCAPULARIS* DURING THEIR OFF-HOST PERIODS

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THE EFFECT OF BIOTIC AND ABIOTIC FACTORS WITHIN THE SOIL ENVIRONMENT ON THE MORTALITY OF *IXODES SCAPULARIS* DURING THEIR OFF-HOST PERIODS

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The blacklegged tick (*Ixodes scapularis*) is the primary vector for multiple widespread tick-borne diseases in the United States, including anaplasmosis, babesiosis, Powassan virus, and Lyme disease. This species spends most of its two-year life cycle either questing for hosts on leaf litter and low-lying vegetation, or inactive during non-questing periods in the soil. Despite the prolonged periods this species spends off-host, the effects of the biotic and abiotic components of the soil ecosystem on the mortality of *I. scapularis* is not well-characterized. I began by exploring the effect of hot, dry weather conditions on Lyme disease incidences in geographic areas with differing endemic histories. In long-term endemic areas the incidence of Lyme disease was significantly lower during hot and dry weather, while in areas that have become endemic for Lyme disease more recently, there was a strong positive linear relationship between time and incidence. Similarly, I demonstrated that hot, dry weather conditions negatively affected the activity of questing *I. scapularis*. Next, I evaluated the efficacy of using microcosms to deploy and recover ticks from the field. This technique allowed me to exclude host-related factors and investigate the direct effects of local-scale site factors on tick survival. These microcosms were used to investigate the effect of weather on overwintering *I. scapularis* nymphs, and also determine the effect of soil-dwelling arthropod predators on the survival of engorged larval and nymphal *I. scapularis*.

The timing of *I. scapularis* mortality events during their overwintering period (September – June) was determined by placing ticks in field microcosms and collecting subsets of the microcosms every 50 days throughout this period. Lipid storage levels were also quantified to determine the body condition of all the ticks that were retrieved from the field. Nymphal *I. scapularis* experienced limited mortality over the winter, with the majority perishing in the spring and early summer. Survival during this period was also positively correlated with tick body condition (i.e. lipid storage). Additionally, these data were compared with those from a previous season during which the weather was relatively cold and wet. Tick survival and energy storage were found to be significantly higher during the cold and wet year, but further investigation was needed as these data were collected on different sites. To further explore the effect of winter weather, snow cover was manipulated over microcosms containing *I. scapularis* nymphs. At the end of the season snow removal was found to have no effect on overwinter tick survival, but there was a negative relationship between tick survival and the number of large arthropod predators in the microcosms.

The prevalence of soil-dwelling arthropod predators which would target *I. scapularis* nymphs and engorged larvae was investigated, both in the laboratory and under field conditions. Overall, it was observed that few species of arthropod predator targeted *I. scapularis*, but those which did showed a preference for engorged larvae over unfed nymphs. An important exception was *Schizocosa ocreata*, a common species of wolf spider, which was found to target *I. scapularis* in the laboratory, and significantly reduced tick survival when added to microcosms under field conditions. I concluded by outlining the future research necessary to further investigate the impact of the soil environment on tick activity and behavior, with the goal of

determining the factors driving the high degree of observed spatio-temporal variability present in many tick borne-disease systems.

BIOGRAPHICAL SKETCH

James Burtis grew up in the New York City area, and his early life revolved primarily around the city. This first began to change during his final year in high school when he was accepted into the Walkabout Program, during which he spent three weeks camping and hiking in the Catskills and the high peaks of the Adirondacks. This was the first time that James had spent a significant amount of time in the forest, and it both terrified and fascinated him. As a result, he began to spend time hiking on his own in the Catskills, Adirondacks, and Green Mountains. This also influenced his decision to attend Bennington College, which was set in rural Vermont. James attended Bennington College as an undergraduate from 2004 – to – 2008, and it was during this time that he developed a deeper interest first in the biological sciences, and later in ecology. These interests were nurtured thanks largely to the patient efforts of two Bennington professors, Drs. Kerry Woods and Elizabeth Sherman. They were the first to introduce James to the concept that science is a dynamic puzzle, rather than a series of established facts, an idea which fascinates him to this day. While at Bennington, he was also fortunate to work at a Wolf Sanctuary in New Mexico, and in the genetics laboratory at the Bronx Botanical Gardens, but most importantly, Bennington is where he met Jessie Miglus, his wife.

His difficult experience designing and carrying out an independent research project for his senior thesis at Bennington made James realize that he lacked many of the technical skills that would be necessary for him to succeed as a graduate student. As a result, he spent the next three years working as a research technician on various projects; gaining experience (and making mistakes) as he went along. During this period, James was extremely fortunate to meet and work for many of the researchers who would later mentor him as a graduate student, while also learning a great deal from the technicians and laboratory managers with whom he worked. Of

particular note are Shannon Duerr and Kelly Oggenfuss in Dr. Richard Ostfeld's laboratory, who patiently taught him how to properly collect data in the laboratory and the field.

After three years working as a technician, James applied to be a graduate student in the Department of Natural Resources at Cornell University working in Dr. Joseph Yavitt's laboratory. At first, James struggled to balance teaching and research as a Master's student, but gradually over time he learned to keep his duties (mostly) in equilibrium. Over the years as a graduate student James has learned a great deal about teaching and developing his own research projects. This process has also allowed him to develop and focus on his own (admittedly odd) research interests, as he began to use his interest in soil ecology to inform his research on tick-borne diseases. Ultimately, this led James to develop a series of research projects focusing on the off-host life of blacklegged ticks. James has truly enjoyed his time as a graduate student, and while it has been trying at times, the friends and connections he and Jessie have made in Ithaca will follow them throughout their lives.

My dissertation is dedicated to my wife Jessie Miglus, and my mother Grace Cosgrove. Without their love, support, and encouragement, I would not be here.

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I first would like to acknowledge all four of the researchers who sit on my committee, each of whom has provided me with invaluable support and guidance over the years. I am indescribably grateful to my advisor, Joseph Yavitt, who has not only provided me with enormous guidance while developing my research projects, but has also always gone out of his way to make himself available whenever I needed advice. Over the years Timothy Fahey has taught me to become a better writer through his detailed editing and comments, which provided me with a blueprint (which I at least try to emulate) for the writing style that I employ as a researcher. I would like to thank Richard Ostfeld for not only introducing me to the complex nature of tick-borne disease systems, but also for acting as a guide while I delved into the broader literature to learn about these fascinating systems. Finally, Patrick Sullivan introduced me to the world of statistics, first through his courses and later as a member of my committee. I did not come to graduate school expecting to develop these skills, much less to genuinely enjoy carrying out statistical analyses (at least most of the time). Once again, I owe each of my committee members and enormous debt of gratitude for their years of guidance, dedication, and support.

I would also like to thank the multitude of students and technicians who assisted me both in the laboratory and the field. I would never have been able to carry out my research without the enormous amount of help I received, both from students in the Fahey / Yavitt laboratory, and technicians in the Ostfeld laboratory. Ultimately, the number of people who have assisted me throughout my Ph.D. is too long to list here, but I would like to thank Kelly Oggenfuss for helping me navigate the Ostfeld laboratory while I was working at the Cary Institute, and of course I must mention Caroline Pflueger, who has been an outstanding laboratory assistant even

during the most difficult field seasons. I have also been fortunate to have the support of an incredible network of friends here in Ithaca. My fellow graduate students, particularly Annise Dobson, Darragh Hare, and Maria Carrizales, have been extremely supportive. I would also like to thank my many non-graduate student friends, particularly Kari Aldrich and Sam Mameli, for forcing me to leave the laboratory and ensuring that I do not meld with my computer. Once again, thank you all for your help and support!

Finally, I would like to thank those who provided financial support for my research, as without this support none of my work would have been possible. I received funding from the Atkinson Center for a Sustainable Future, the Bentley-Holden Fund, and the Kieckhefer Adirondack Fellowship program. These programs allowed me to hire assistants, purchase lab equipment, and live at the Cary Institute of Ecosystem Studies for several summers while carrying out my research. Thank you all again for your generous support.

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CHAPTER 1

THE IMPACT OF TEMPERATURE AND PRECIPITATION ON BLACKLEGGED TICK ACTIVITY AND LYME DISEASE INCIDENCE IN ENDEMIC AND EMERGING REGIONS[1]

Abstract

The incidence of Lyme disease shows high degrees of inter-annual variation in the northeastern United States, but the factors driving this variation are not well understood. Complicating matters, it is also possible that these driving factors may vary in regions with differing histories of Lyme disease endemism. We evaluated the effect of the number of hot (T > 25° C), dry (precipitation = 0) days during the questing periods of the two immature *Ixodes scapularis* life stages (larval and nymphal) on inter-annual variation in Lyme disease incidence between 2000 and 2011 in long-term endemic versus recently endemic areas. We also evaluated the effect of summer weather on tick questing activity and the number of ticks found on small mammals between 1994 and 2012 on six sites in Millbrook, NY. The number of hot, dry days during the larval period of the previous year did not affect the human incidence of Lyme disease or the density of questing nymphs the following season. However, dry summer weather during the nymphal questing period had a significant negative effect on the incidence of Lyme disease in the long-term endemic areas, and on the density of questing nymphs. Summer weather conditions had a more pronounced effect on actively questing *I. scapularis* collected via

[1] Burtis JC, Sullivan P, Levi T, Oggenfuss K, Fahey TJ, and Ostfeld RS. 2016. The impact of temperature and precipitation on blacklegged tick activity and Lyme disease incidence in endemic and emerging regions. Parasites & vectors. 9(1): e606. https://doi.org/10.1186/s13071-016-1894-6.

dragging than on the number of ticks found feeding on small mammals. In recently endemic areas Lyme disease incidence increased significantly over time, but no trend was detected between disease incidence and dry summer weather. Recently endemic regions showed an increase in Lyme disease incidence over time, while incidence in long-term endemic regions appears to have stabilized. Only within the stabilized areas were we able to detect reduced Lyme disease incidence in years with hot, dry summer weather. These patterns were reflected in our field data, which showed that questing activity of nymphal *I. scapularis* was reduced by hot, dry summer weather.

Introduction

Lyme disease is the most common vector-borne disease in the United States (Adams et al. 2013). Many studies have attempted to improve our understanding of the factors driving its spread and amplification in new areas (Tran & Waller 2013, Turney et al. 2014) as well as the habitat suitability of new areas in the United States and Canada for *Ixodes scapularis* (Brownstein et al. 2003, Diuk-Wasser et al. 2006, Ogden et al. 2006), the primary vector for the Lyme disease spirochete (*Borrelia burgdorferi*). Correlative models have explored the effect of climatic factors on Lyme disease incidence averaged across expansive geographic regions, most commonly states in the U.S.A. (Subak 2003, McCabe & Bunnell 2004). Unfortunately, there are large variations in incidence within many states (Eisen et al. 2016a), and the factors that drive inter-annual variation in Lyme disease incidence vary spatially (Eisen et al. 2016b). Specifically, underlying factors such as physician and public awareness have a strong impact on the reporting of Lyme disease cases in emerging areas (Freimuth et al. 2006, Ogden et al. 2009), and data collected at a coarse scale (State) may not reveal patterns that exist at finer scales (County).

Climate and weather conditions are likely to play a role in the incidence of Lyme disease because the demography and behavior of *I. scapularis* are sensitive to variation in temperature and precipitation (McCabe & Bunnell 2004, Ogden et al. 2004). However, few studies have attempted to connect these weather and climate effects to patterns in the human incidence of Lyme disease (Eisen et al. 2016b). There is strong evidence that Lyme disease and tick populations are spreading from two epicenters in the United States, one in the upper Midwest, and another the Northeast (Hoen et al. 2009, Margos et al. 2012). Some have argued that the geographic spread of Lyme disease is caused, at least in part, by climate warming trends (Beard et al. 2016). However, other factors might contribute to the spread of Lyme disease, including

facilitation of the expansion of *I. scapularis* populations by vertebrate hosts (Mathers et al. 2011), and amplification of *B. burgdorferi* infection through vector and host communities (Keesing et al. 2010, Vuong et al. 2014). Possible effects of climate and weather on disease incidence in these newly emerging areas may be difficult to detect due to these confounding factors. In contrast, the effects of weather and climate on Lyme disease incidence in areas that were invaded decades ago might be less subject to confounding variables. Differences in the focal area under study (expanding vs. long-term endemic regions) may explain some of the variation in results between studies that have explored the effect of weather and climate on inter-annual variation in human cases of Lyme disease (Ostfeld & Brunner 2015).

The effect of specific climatic variables on *I. scapularis* density, survival, and behavior has been thoroughly investigated (Bertrand & Wilson 1996, Schulze et al. 2001, Brunner et al. 2012, Hayes et al. 2015, Burtis et al. 2016). The effect of relative humidity on *I. scapularis* survival is well documented, with significantly reduced survival in low humidity environments (Needham & Teel 1991, Stafford 1994). Duration of exposure to dry conditions is an important factor in determining *I. scapularis* mortality, with longer periods of exposure leading to significantly higher rates of mortality (Rodgers et al. 2007). Many species of tick will modify their behavior to avoid desiccation (Randolph 2014). One of the most commonly observed behaviors is that *I. scapularis* will quest at a lower height when temperatures are high and relative humidity is low (Schulze et al. 2001), probably because of the need to descend to moist microhabitats for rehydration. This lower questing height reduces the probability that ticks will come into contact with large vertebrate hosts (Lefcort & Durden 1996, Prusinski et al. 2006), including humans. The effect of atmospheric saturation deficit on tick behavior has been studied in *I. ricinus* (Randolph & Storey 1999), a tick species that is closely related to *I. scapularis* and

is the primary vector for Lyme disease in Europe. Atmospheric humidity has the strongest impact on *I. ricinus* behavior when temperatures are greater than 24° C (MacLeod 1935, Perret et al. 2000). Furthermore, *I. scapularis* questing behavior peaks at 25° C with lower levels of activity as the temperature increases (Vail & Smith 1998). Berger et al. (2014) found that low relative humidity reduces the seasonal activity of *I. scapularis* nymphs. They also found that early season dry periods could lead to reduced late-season tick populations. We expanded on this research by exploring the effect of summer climate on tick density with an additional metric, small mammal body burdens, and by exploring the effect of summer weather on the human incidence of Lyme disease across a broad region.

We analyzed data collected by the Centers for Disease Control and Prevention (CDC) for annual Lyme disease incidence by county, and a long term field dataset for tick densities collected at the Cary Institute of Ecosystem Studies (Ostfeld et al. 2006). We explored the effect of the number of hot (T > 25° C) dry (precipitation = 0) days on the human incidence of Lyme disease and *I. scapularis* activity. We targeted our analyses for the activity peaks of two *I. scapularis* life stages; 1) May through July for nymphs (2^{nd} instar), and 2) August through September for larvae (1^{st} instar). The nymphal life stage is responsible for the majority of human infections (Pepin et al. 2012), and recruitment and survival of the larval stage is likely to have a strong effect on the number of nymphs emerging the following year (Ostfeld et al. 2006, Estrada-Peña A & Estrada-Sánchez 2014). Additionally, we explored whether the effect of summer weather on inter-annual variation in Lyme disease incidence was consistent between areas with a long history of endemic Lyme disease, and those that have become endemic more recently. We hypothesized that weather effects would be more evident in long-term endemic areas, defined as

those areas that have the longest history of reported Lyme cases (Eisen et al. 2016a, Ciesielski et al. 1988, Ciesielski et al. 1989).

We also used field measurements to distinguish whether the effect of summer weather conditions on *I. scapularis* is behavioral, or demographic, affecting long-term survival rates. Behavioral effects could manifest as either lower overall questing activity, lower questing height, or a combination of the two. We used two methods to distinguish between weather effects on questing activity versus questing height. If hot, dry weather reduces questing height, we expected to find reduced numbers of questing *I. scapularis* nymphs captured with the tick dragging method as this method is most efficient when nymphal questing height is high (Vail & Smith 2002). In contrast, if hot, dry weather reduces overall questing activity, we would expect that the number of ticks feeding on small mammals, which are sampling ticks at ground level, would be reduced (Prusinski et al. 2006). If the number of hot, dry days during the previous year's larval questing period reduces nymphal tick densities or Lyme disease incidence the following summer, then we would conclude that the long-term demographic effects of summer weather are important. Both behavioral and demographic effects can impact human health, but the implications for the long term effect of climate change on tick-borne diseases may differ.

Methods

Lyme disease data

The annual numbers of Lyme disease cases were reported by county between 2000 and 2011 by the CDC (CDC 2016a). Lyme disease cases are generally under-reported by the CDC (Nelson et al. 2015). In an effort to address under-reporting the CDC altered their reporting standards in 2008, broadening the criteria for reportable cases. To account for possible under-reporting bias we have included the CDC's reporting type as a factor in our analyses. Annual

Lyme disease case counts are available for all United States counties, but cases are reported in the patient's county of residence, so in order to reduce reporting errors due to patient travel we focused on the northeastern United States where local incidences are high and > 80% of cases are reported. We split these data into two groups; 1) a long-term endemic region (Eisen et al. 2016a) which includes the Hudson Valley of New York, southern New England, and northern New Jersey (Fig. 1.1b), and 2) a more recently endemic region, which includes the rest of the counties in the northeastern region (Fig. 1.1a). Island counties were excluded from our analyses. Comparisons between these regions allowed us to explore how factors affect inter-annual trends in Lyme disease incidence in regions with presumed stabilized (long-term endemic), and increasing (recently endemic) Lyme incidence. Lyme disease case counts were corrected for county populations using annual county population estimates collected by the United States Census Bureau (USCB 2016). All Lyme disease incidence data are presented as the number of cases per county per 100,000 residents.

Body burden on small mammals

Measurements of the body burdens for *I. scapularis* nymphs and larvae on chipmunks (*Tamias striatus*) and white-footed mice (*Peromyscus leucopus*), respectively, provided metrics for the number of ticks feeding on small mammals. Field data were collected in a long term study at the Cary Institute of Ecosystem Studies in Millbrook, Dutchess County, NY (41°47'1.04"N; 73°43'56.49"W). The field sites and methods are described in detail in Ostfeld et al. (Ostfeld et al. 2006). These data were collected annually from 1994 to 2012 on six small mammal trapping grids. Each trapping grid contained 242 Sherman traps arranged in pairs on an 11 x 11 grid, with 15 m spacing between each trapping station, and each grid covering approximately 2.25 ha. Small mammals were trapped on each grid for two consecutive nights, multiple times between

April and November each year. Additional details regarding the small mammal data collection methods are described in Levi et al. (2015).

The number of larvae and nymphs were counted on the head and neck of each animal the first time they were caught in a trap. Counting nymphs on the heads of chipmunks provides a reliable metric for their body burdens (number of attached ticks per chipmunk), while counts for the number of larvae on white-footed mice provides a reliable estimate of their total body burden (Levi et al. 2015). We calculated the annual average body burden of chipmunks during the *I. scapularis* seasonal nymph peak, and the annual average body burden of white-footed mice during the larval peak. These served as metrics for the number of nymphs and larvae feeding on animal hosts in a given year. The number of larvae counted on the heads of chipmunks, and nymphs counted on the heads of mice did not provide reliable metrics for the animal's total body burdens, so these data were not used for our analyses (Levi et al. 2015). All data collected on the six grids were combined to represent an overall annual average body burden for mice (larvae), and chipmunks (nymphs). All small mammal handling procedures were approved by the Cary Institute of Ecosystem Studies IACUC.

Density of questing nymphs

The density of questing nymphs was measured at the Cary Institute of Ecosystem Studies during the same time period (1994 – 2012) and on the same six sites as the small mammal body burden data. Actively questing *I. scapularis* nymphs were collected using a dragging method whereby a 1 m^2 white corduroy cloth is dragged along the surface of the leaf litter and understory vegetation. All sites were sampled multiple times during the nymphal peak each year (May – Jul). The total area covered for each sample was 450 m^2. Drag cloths were checked for ticks every 30 m and the number of *I. scapularis* nymphs was recorded. We averaged the density of *I.*

scapularis nymphs during their activity peak across all six sites to estimate an overall density of nymphs (DON) for each year.

Temperature and precipitation data

County-wide temperature and precipitation data were collected from the CDC's Wide-ranging Online Data for Epidemiological Research (WONDER) database (CDC 2016b). The WONDER database provides climate data from National Oceanographic and Atmospheric Administration (NOAA) weather stations in the United States, which can be averaged by various administrative boundaries. We used the mean of all daily temperature and precipitation data collected by weather stations within county boundaries to calculate our parameters. Daily maximum temperatures and daily precipitation were retrieved from the data base for the *I. scapularis* nymph questing period (May – Jul) for the year the Lyme disease cases were reported. We also retrieved temperature and precipitation data for the previous year's larval questing period (Aug – Sep). We then calculated a cumulative measure for the number of days where T > 25° C and precipitation = 0 during the previous year's larval questing period, and the current year's nymphal questing period. This provided us with metrics for the number of hot, dry days (HDD) during the questing periods of the two immature *I. scapularis* life stages. We abbreviated the metric for the nymphal questing period as N-HDD, and the metric for the larval questing period during the preceding year was L-HDD. Although ideally atmospheric humidity data would be used for this purpose, no consistent daily records exist at the county scale for our study period.

We also compared the field data on tick abundance collected at the Cary Institute of Ecosystem Studies against the CDC WONDER data for Dutchess County between 1994 and 2011. We had one additional year of field data (2012) which was not included in the CDC

WONDER database. To calculate N-HDD for 2012 we accessed the NOAA database which is used to calculate the CDC WONDER data, and downloaded weather station data for stations within Dutchess County to calculate maximum temperature and precipitation for 2012 N-HDD.

Data analyses

We used two mixed effects generalized additive models to explore whether time was a linear estimator for Lyme disease incidence, first in our long-term endemic subset which included the Hudson Valley, Southern New England, and northern New Jersey, and then in the recently endemic subset which included the remaining counties in the northeastern region. Lyme disease incidence is strongly spatially correlated at this scale, so both models included a smoothing term for interactions between latitude and longitude to account for the spatial variation in incidence. The CDC's reporting metric (which changed in 2008) was also included in both models, as was county as a random effect. County was included as a random effect because observations within the same county are not independent. These factors de-trended the data, and will be referred to hereafter as the de-trending model. We used the de-trending models to explore whether Lyme disease incidence was increasing over time in our two data subsets (long-term endemic and recently endemic) (Fig. 1.1). We also used these models to explore the effect of summer temperature and precipitation during *I. scapularis* questing periods (L-HDD and N-HDD) on inter-annual variation in our long-term and recently endemic subsets. F-tests and Bayesian information criterion (BIC) values were used to compare models and evaluate the addition of new factors using the procedures described in Zuur et al. (2009). Lyme disease incidence data were log transformed for all analyses.

We used three linear models to explore the effect of summer temperature and precipitation (L-HDD and N-HDD) on the body burdens of small mammals. The first model

included L-HDD compared against the average annual larval body burden of mice in the same year, the second compared L-HDD against the nymphal body burden of chipmunks the following year, and the third compared N-HDD against the nymphal body burden of chipmunks in the same year. All three models included the number of mice or chipmunks caught that year as a covariate. These models allowed us to explore whether summer climate affected the number of ticks feeding on small mammals, and whether that effect carried over from the previous year.

Additionally, two linear models explored the effect of summer temperature and precipitation on the density of questing *I. scapularis* nymphs (DON) using the dragging data collected at the Cary Institute of Ecosystem Studies. The first model compared L-HDD against DON to explore whether the weather during the previous year's larval questing period affected the number of actively questing nymphs the following year. The second model compared N-HDD against DON to see if summer weather during that year affected nymphal questing activity. These models also included the number of chipmunks caught that year as a factor. Chipmunk count was included as they act as the primary host for nymphs, and chipmunk population density can affect DON (Levi et al. 2015). All analyses were performed in R version 3.2.3.

Results

In both the long-term and recently endemic regions inclusion of a smoothing term for latitude and longitude, CDC reporting type, and county as a random effect improved the models based on their BIC scores. Overall, the recently endemic region showed a significant increase in Lyme disease incidence between 2000 and 2011 ($t = 13.48$; $df = 2210$; $P < 0.001$) (Table 1.1) (Fig. 1.1a), while time was not a significant linear predictor of Lyme disease incidence in the long-term endemic region (Table 1.2) (Fig. 1.1b). We examined the effect of the number of hot ($T > 25°$ C), dry (Precip = 0) days during the previous year's larval questing period (L-HDD)

and during the current year's nymphal questing period (N-HDD) on the incidence of Lyme disease in each region in de-trending models (Table 1.3). L-HDD did not affect the incidence of Lyme disease in either the long-term (t = -0.52; df = 396; P = 0.60) (Fig. 1.2a), or the recently (t = 0.24, df = 2209, P = 0.82) endemic regions. This conclusion (Fig. 1.2) is based on the analysis of residuals of the de-trending model for the long-term endemic region plotted against N-HDD and L-HDD. N-HDD had a significant negative effect on the incidence of Lyme disease (t = -5.48; df = 396; P < 0.001) in the long-term endemic region (Fig. 1.2b). N-HDD had no significant effect on Lyme disease incidence in the recently endemic region (t = -0.01, df = 2055, P = 0.33). The lack of effect of L-HDD on Lyme disease incidence in both regions is also reflected in their BIC scores (Table 1.3).

L-HDD did not affect the density of questing nymphs (DON) the following year (t = -0.46; df = 1, 16; P = 0.65) (Fig. 1.3a). On the other hand, N-HDD had a significant negative effect on DON (t = -2.60; df = 1, 16; P = 0.02) (Fig. 1.3b). The number of chipmunks caught annually over a field season had a significant negative effect on DON in both the L-HDD (t = -2.57; df = 1, 16; P = 0.02), and N-HDD (t = -2.69; df = 1, 16; P = 0.02) models.

Summer weather during the questing periods of *I. scapularis* larvae did not affect the average number of larval *I. scapularis* found on mice that year (t = -1.56; df = 1, 16; P = 0.14), or the number of nymphs found on chipmunks the following year (t = -0.21; df = 1, 16; P = 0.83) (Fig. 1.4a). N-HDD did not have a significant effect on the average *I. scapularis* nymphal body burdens of chipmunks (t = -1.82; df = 1, 16; P = 0.09) (Fig. 1.4b). The number of chipmunks caught during a given field season had a significant negative effect on the number of nymphs found on chipmunks for both the L-HDD (t = -2.70; df = 1, 16; P = 0.02) and N-HDD (t = -2.76; df = 1, 16; P = 0.01) models. There was a marginally significant negative correlation between the

number of mice caught in a field season and the average larval body burden of mice (t = -2.09; df =1, 16; P = 0.05).

Discussion

Lyme disease incidence and region

The significant increase in the incidence of Lyme disease in counties in the northeastern United States with a recent endemic history of Lyme disease (Fig. 1.1a) supports the observation that Lyme disease is spreading rapidly in the United States, with many new areas becoming endemic (Eisen et al. 2016a), and showing steadily increasing infection levels (Bacon et al. 2008). On the other hand, in areas with a long-term endemic history the incidence of Lyme disease appears to have stabilized at least since 2000 (Fig. 1.1b). Thus, a threshold for Lyme disease incidence appears to exist, above which incidence stabilizes. After this threshold is reached, inter-annual variation in the incidence of Lyme disease may be driven by a different suite of factors that affect the inter-annual variation in tick populations (Pepin et al. 2012), such as weather (Schulze et al. 1997, Hayes et al. 2015), or host abundance and diversity (Jones et al. 1998, LoGiudice et al. 2003, Ostfeld et al. 2006).

In newly emerging areas factors such as physician awareness, human behavior (Freimuth et al. 2000, Ogden et al. 2009), and the amplification of *B. burgdorferi* in host communities (Keesing et al. 2010, Vuong et al. 2014) may be the dominant factors affecting the recorded human incidence of Lyme disease. When Lyme disease has recently spread to a new area a considerable lag in notifying the public and modifying human behavior may cause an increase in incidence (Estrada-Peña & Jongejan 1999, Lane et al. 2004). Physicians may also initially report a high number of cases when Lyme disease is emerging, and become less aggressive in their reporting as it becomes more commonplace (Bacon et al. 2006, Nelson et al. 2015). Additionally,

Lyme disease is amplified through the natural reservoir and vector communities as new susceptible individuals become infected. During this amplification period it is possible that the inter-annual effects of summer weather on Lyme disease incidence is obscured by the overall increase in pathogen transmission. Detection of weather effects therefore may be more difficult in areas with newly emerging tick borne diseases.

It is unlikely that all counties in the region will stabilize at the same level of Lyme disease incidence. Factors such as human population density, availability of suitable tick habitat, and human contact rates can affect both *I. scapularis* densities and Lyme disease risk (Maupin et al. 1991, Dister et al. 1997, Allan et al. 2003). Additionally, physicians often under-diagnose diseases once the disease is fully established in a new region (Bacon et al. 2006) and the under-reporting of Lyme disease cases by physicians in the United States is well-documented (Nelson et al. 2015), which further obfuscates the measurement of this threshold. These factors are all likely to vary among counties, affecting the threshold at which Lyme incidence stabilizes. Further research is needed to explore how these factors might interact to affect the stabilization of Lyme disease incidence in different locations.

The effect of N-HDD on Lyme disease incidence and DON

We found that weather conditions during the questing period of *I. scapularis* nymphs (N-HDD) affected county-wide incidences of Lyme disease in the long term endemic region; hot dry summers were associated with significantly reduced incidences of Lyme disease. In contrast, this pattern was not detected in the counties that have more recently become endemic for Lyme disease. This observation supports the assertion that other (non-weather related) factors could mask the effects of weather on inter-annual variation in areas where the disease is newly emerging. Thus, some of the discrepancies that have been observed between studies of inter-

annual variation in Lyme incidence in the northeastern United States may be attributed to the length of time since the emergence of Lyme disease in each location (Ostfeld & Brunner 2015). It is notable that the suppressive effect of hot, dry weather was detected despite the fact that human behavior strongly impacts Lyme disease incidence (Connally et al. 2006), and people spend more time outside during dry weather (Li & Lin 2012), potentially increasing overall contact rates with ticks. Our detection of a relationship between summer weather conditions and Lyme disease incidence in the long-term endemic region, despite a variety of likely confounding factors, suggests a strong effect of summer weather conditions on *I. scapularis* questing behavior. The effect of weather conditions during the nymphal questing period on Lyme disease incidence may be explained in part by the geotropic response of questing nymphs to drought; when conditions are warm and dry ticks quest at lower heights (Vail & Smith 2002), likely reducing contact rates with humans.

Exploration of trends in the field data for the density of questing *I. scapularis* nymphs collected between 1994 and 2012 supports this hypothesis. We found that there was a strong negative correlation between N-HDD and DON (Fig. 1.3b), likely due to the effect of vapor pressure deficit on tick activity (Perret et al. 2000). Field evidence suggests that when conditions are hot and dry *I. scapularis* alters its behavior, reducing its questing height (Schulze et al. 2001). Moreover, laboratory evidence also indicates that relative humidity affects *I. scapularis* questing height (Vail & Smith 2002). This change in behavior is likely to affect contact rates between large animals (including humans) and ticks.

The effect of L-HDD on Lyme disease incidence and DON

We found no significant relationship between the weather conditions during the previous year's larval *I. scapularis* questing period (L-HDD) and inter-annual variation in Lyme disease

incidence in either region (long-term endemic / recently endemic). Nor did we observe any effect on the density of questing nymphs (DON) the following year. Furthermore, there was no relationship between summer weather conditions and the number of larvae found feeding on mice. This is important as the number of larvae successfully feeding on hosts would have a strong impact on nymph populations the following year (Levin & Fish 1998, Ostfeld et al. 2006). This lack of inter-annual effect was also observed by Berger et al. (2014) where weather conditions during previous years did not affect tick densities in the current year. The absence of trends carrying over from the previous summer suggest that the effect of hot, dry summers on tick densities is behavioral, or possibly demographic in the short term, with its effect on Lyme disease incidence restricted to the current season. Overall, we found no evidence of any long-term effect of summer weather on tick populations as a whole, despite a strong short-term effect on the human incidence of Lyme disease and *I. scapularis* nymphal questing activity. Perhaps other factors play a stronger role in the overwinter survival and molting success of larval *I. scapularis*, including variation in host populations (Ostfeld et al. 2006), or winter precipitation events (Hayes et al. 2015, Burtis et al. 2016), among others.

DON and small mammal body burdens

Tick activity or density was significantly reduced during hot dry summers (Fig. 1.3b), but this trend was far weaker in our analysis of small mammal body burdens (Fig. 1.4b), probably because of the differential effect of tick behavior on these two metrics of tick abundance. Tick dragging collects actively questing ticks with a relatively high questing height, and is a good measure of entomological risk for humans (Stafford et al. 1998). This metric is affected by tick questing height because ticks must quest at or above the surface of the leaf litter to come into contact with the collection cloth (Schulze et al. 1997). On the other hand, the number of ticks

counted on small mammal hosts is not as sensitive to changes in tick behavior, and feeding success has a strong effect on tick survival (Levin & Fish 1998, Ostfeld et al. 2006). However, counts of mean tick burdens on hosts can be affected by other factors such as variation in host densities (Levi et al. 2015), and density-dependent effects whereby tick feeding success is reduced when host body burdens increase (Levin & Fish 1998), although the latter is not consistently observed (Hazler & Ostfeld 1995). The fact that these two methods appear to suggest differing trends may indicate that the impact of hot dry summers on *I. scapularis* is an ephemeral behavioral effect, causing them to quest at lower heights, and may not directly affect tick populations or long-term trends in the incidence of Lyme disease. More research is needed regarding the cumulative effect of long-term drought on *I. scapularis* survival and energetics. Furthermore, the variable results found with differing metrics highlights the importance of using multiple metrics when studying complex vector-borne disease systems.

Conclusions

Hot, dry weather reduces the density of questing *I. scapularis* nymphs, but does not appear to have a strong impact on the density of *I. scapularis* larvae or nymphs feeding on small mammals. Weather conditions in one summer do not appear to have carryover effects on tick density the following summer. We conclude that summer weather conditions affect *I. scapularis* questing behavior, but may not have strong demographic effects. Reduced density of questing *I. scapularis* nymphs and behavioral effects during hot, dry periods appears to reduce the incidence of Lyme disease in the human population in areas with a long Lyme disease endemic history (long-term endemic region). Similar suppression of nymph density and Lyme disease incidence in hot, dry summers was not detected in areas where Lyme disease is invading (recently endemic region). We suspect that these weather effects are masked in recently endemic areas experiencing

directional changes in tick populations, host communities, or human recognition of Lyme disease risk. These regional differences should be taken into account in future modeling attempts.

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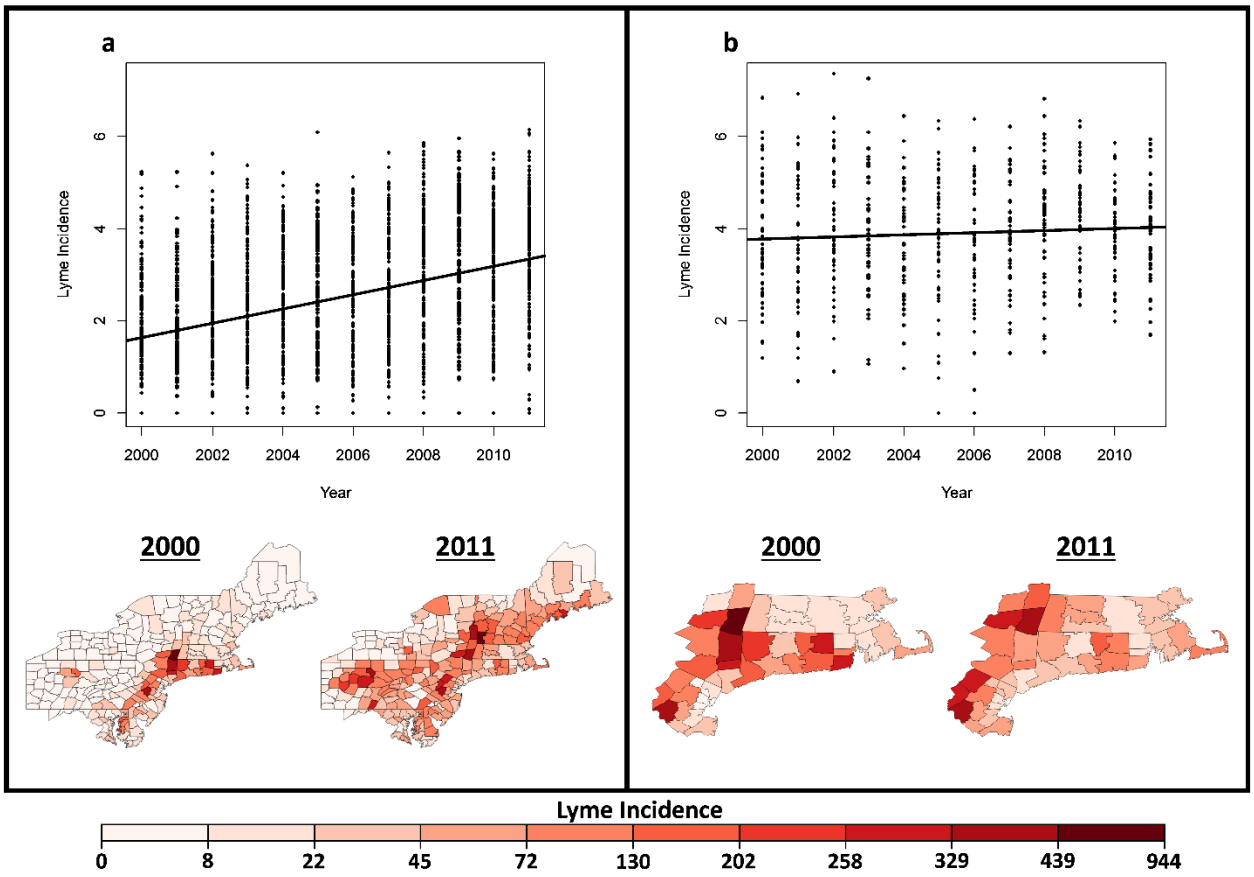

Figure 1.1: a) The incidence of Lyme disease in the recently endemic region over time. The scatterplot shows the log of the incidence as time progresses from 2000 to 2011 in the recently endemic region, while the maps show the incidence at the starting point (2000), and ending point (2011) for our entire dataset. b) The incidence of Lyme disease in the counties of our subset of long-term endemic counties in the Hudson Valley of New York, southern New England and northern New Jersey. The legend represents the incidence of Lyme disease per 100,000 people and the color scheme applies to all four maps.

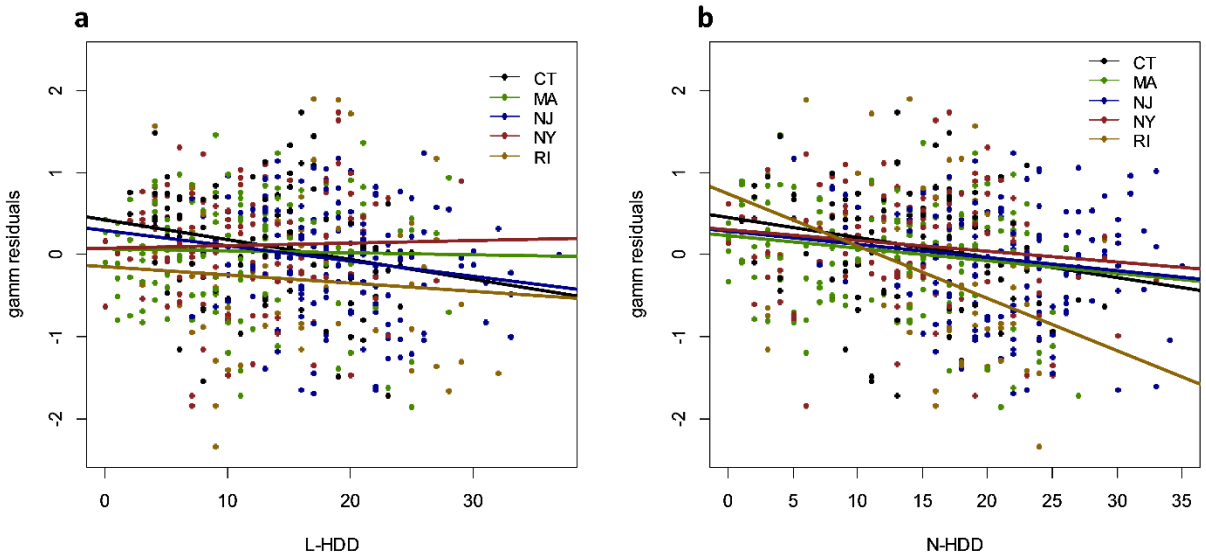

Figure 1.2: a) The residuals of the de-trending generalized additive mixed model (gamm) for the long-term endemic region plotted against L-HDD, and b) the residuals of the de-trending gamm plotted against N-HDD. Best fit lines are included for each of the five states (Connecticut, Massachusetts, New Jersey, New York, and Rhode Island) included in the model. The de-trending models included CDC reporting type, a smoothing term for latitude and longitude, and county as a random effect.

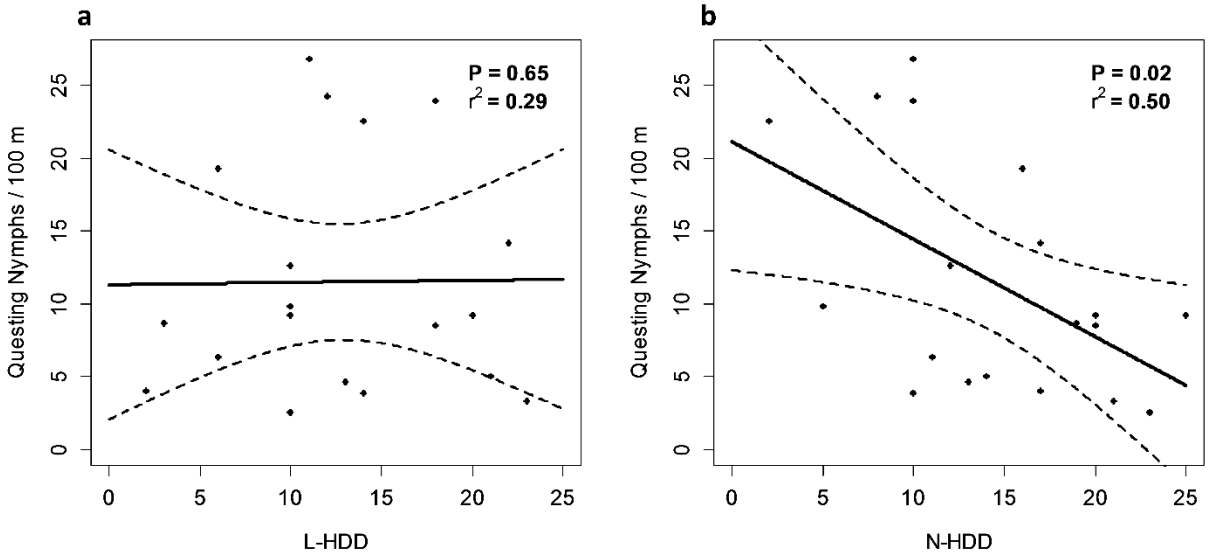

Figure 1.3: a) The density of questing nymphs (DON) per 100 m^2 determined via drag sampling compared against L-HDD, and b) DON compared against N-HDD. Nymphs show lower activity during hot dry summers, but there is no effect of weather from the previous year. P-values and r^2 statistics are based on models including both the weather parameters and small mammal counts.

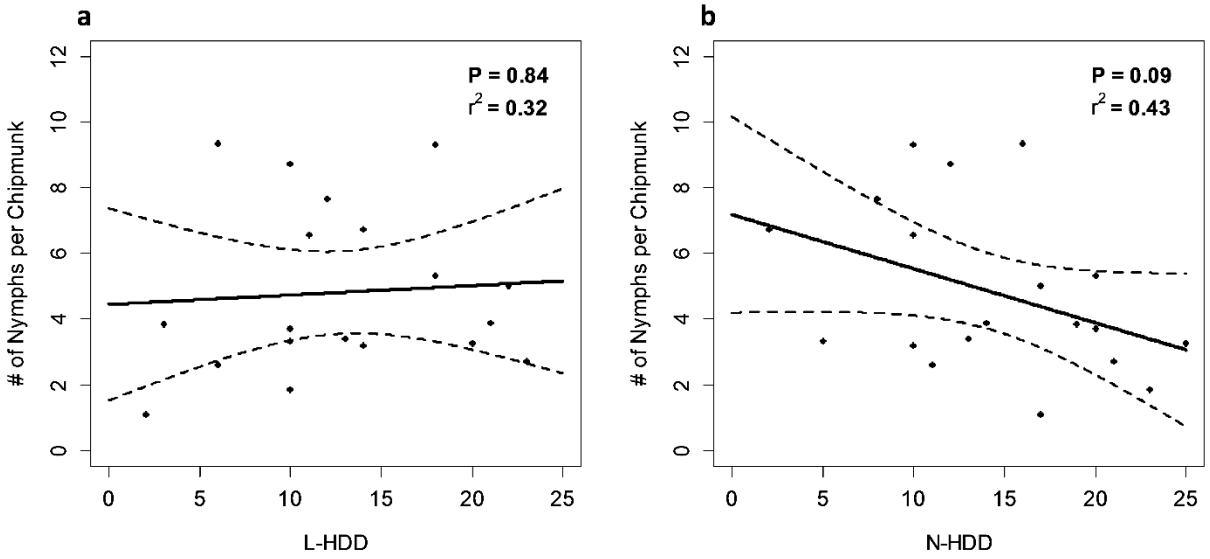

Figure 1.4: a) The average body burden of chipmunks during the peak of *I. scapularis* nymph activity plotted against L-HDD, and b) the average body burden of chipmunks during the *I. scapularis* nymph activity peak plotted against N-HDD. P-values and r^2 statistics are based on models including both the weather parameters and small mammal counts. Neither L-HDD nor N-HDD had a significant effect on the number of nymphs found on chipmunks.

Table 1.1: The F-test statistics for all the fixed effects included in the generalized additive mixed models for the recently endemic region. County was included as a random effect in this model. * Denotes a factor which significantly improved the model according to the BIC value.

Recently Endemic Region			
Parameters	**DF**	**F**	**p-value**
Reporting Type	1	43.03	< 0.001*
Year	1	175.02	< 0.001*
Latitude x Longitude	20	14.16	< 0.001*
N-HDD	1	3.67	0.06
L-HDD	1	0.12	0.73
Residual Degrees of Freedom = 2219, r^2 = 0.52			

Table 1.2: The F-test statistics for the parameters included in the generalized additive mixed models for the long-term endemic region. County was included as a random effect in this model. * Denotes a factor which significantly improved the model according to the BIC value.

Long-Term Endemic Region			
Parameters	**DF**	**F**	**p-value**
Reporting Type	1	11.44	< 0.001*
Year	1	0.00	0.99
Latitude x Longitude	17	6.21	< 0.001*
N-HDD	1	21.38	< 0.001*
L-HDD	1	3.37	0.07
Residual Degrees of Freedom = 565, r^2 = 0.67			

Table 1.3: Bayesian information criterion (BIC) values for the generalized additive mixed models run for the long-term and recently endemic regions exploring the effect of summer temperature and precipitation during the previous year's larval questing period (L-HDD), and that year's nymphal questing period (N-HDD). The de-trending models included CDC reporting type, a smoothing term for latitude and longitude, and county as a random effect. BIC values show model improvement for L-HDD, N-HDD, and year when added to the de-trending models. Bolded values indicate a significant improvement.

Model	De-trending Model	Year	L-HDD	N-HDD
Long-term Endemic Region	1140.1	1144.5	1141.7	**1122.4**
Recently Endemic Region	5976.0	**5818.1**	5825.7	5822.1

CHAPTER 2

METHOD FOR THE EFFICIENT DEPLOYMENT AND RECOVERY OF *IXODES SCAPULARIS* (ACARI: IXODIDAE) NYMPHS AND ENGORGED LARVAE FROM FIELD MICROCOSMS[2]

Abstract

Factors affecting the survival of *Ixodes scapularis* Say during diapause are poorly known. This is partially due to the difficulty involved in collecting ticks which are not actively questing. A possible method to overcome this issue involves the use of microcosms containing litter material and soil, but an effective method for tick recovery is required. This study tested three methods for the recovery of *I. scapularis* nymphs from soil microcosms during their active and inactive periods, as well as recovery of engorged larval *I. scapularis*. The first method was hand sorting for 120 minutes; the second was sorting for 30 minutes before placing the contents of the microcosm into a Berlese funnel for 72 hours; and the third method was placing the microcosm contents into a Berlese funnel for 72 hours with no prior hand sorting. Hand sorting alone and the combination of hand sorting plus the Berlese funnel were the most effective recovery methods for both active nymphs and those in diapause. Hand sorting alone was not an effective method for the recovery of engorged larvae and Berlese funnel extraction alone was not the most effective method for any of the *I. scapularis* physiological states tested. Overall, a combination of hand sorting and Berlese extraction was an effective recovery method for all physiological

[2] Burtis JC. 2017. Method for the efficient deployment and recovery of *Ixodes scapularis* (Acari: Ixodidae) nymphs and engorged larvae from field microcosms. Journal of Medical Entomology. 54(6): 1778-1782. https://doi.org/10.1093/jme/tjx157.

states and was 58.3% more time-effective compared against hand-sorting alone. This method will allow researchers to process microcosm samples effectively and efficiently, improving our ability to investigate the ecology of *I. scapularis* during their inactive periods.

Introduction

It is important to understand local-scale patterns in the distribution of *Ixodes scapularis* Say given their important role as a vector of multiple common tick-borne diseases in North America. Much of our knowledge is currently based upon data collected during the time when *I. scapularis* is actively questing. Understanding the patchy spatial patterns of *Ixodes scapularis* densities at a local scale (Pardanani & Mather 2004) requires increased study of the direct impact of site-specific factors on *I. scapularis* survival, particularly during their inactive periods. Many tick collection techniques require ticks to be actively questing and are affected by tick behavior and host density (Schulze et al. 1997, Levin & Fish 1998). Few studies have investigated how specific conditions affect the survival of inactive *I. scapularis* while allowing them access to their natural soil refugium under field conditions (Lindsay et al. 1995, Ginsberg et al. 2004, Bertrand & Wilson 1996). These inactive (non-questing) periods make up the majority of the *I. scapularis* life cycle (Ostfeld & Brunner 2015), and thus conditions affecting tick survival over this time period are likely to play an important role in overall rates of survival.

The most common methods for the quantification of tick populations under variable environmental conditions are 1) the dragging method, or 2) measurement of host body burden (Simon et al. 2014, Johnson et al. 2016). Similarly, data from these methods are also used to model the potential geographic range expansion of *I. scapularis* (Brownstein et al. 2003, Hahn et al. 2016). Data collected via these methods provide reliable measures of *I. scapularis* questing activity and entomological risk for humans, but can be affected by confounding factors such as weather and host density (Schmidt et al. 1999, Schulze & Jordan 2003, Brunner & Ostfeld 2008). Interference from these factors makes it difficult to separate the effects of dispersal, collection conditions (e.g. temperature or relative humidity), and host populations from other factors that

may directly affect mortality, such as microhabitat availability or weather and climatic conditions (Ginsberg et al. 2004). These confounding factors may also partially account for the high degree of variability observed in tick density data (Ostfeld et al. 2006, Hayes et al. 2015).

An alternative method for quantifying tick survival involves building microcosms containing soil and litter material from the field that provides realistic refugium for inactive ticks (Brunner et al. 2012, Burtis et al. 2016a). Escape rates from this style of microcosm have not been formally evaluated, nor have recovery methods for different life stages or physiological states of *I. scapularis*. This is important because questing ticks may be easier to recover by hand than those collected during their diapause period. Engorged *I. scapularis* will also be difficult to recover by hand as they will not be searching for hosts, and are difficult to visually differentiate from the soil. These behavioral differences may affect recovery rates, which ultimately affects estimates for the mortality or survival of different life stages in the field in microcosm experiments.

Information is presented here pertaining to the construction of microcosms containing soil and leaf litter from the field, and the evaluation of three different methods for the recovery of *I. scapularis*, in three different physiological states (engorged larvae / nymphs in diapause / actively questing nymphs). High recovery rates of actively questing nymphs when hand sorting were expected, while nymphs in diapause and engorged larvae should be most effectively recovered via methods using Berlese funnels.

Methods

Microcosm construction

Microcosms were constructed using PVC cores (15 cm diameter and 5 cm height) with four holes (2 cm diameter each) drilled around the edge. The additional holes were added to

mitigate the potential insulating effect of the PVC segment. Fine mesh nylon organdy bags (15 cm x 20 cm) with two sides tightly stitched together were constructed. The top was left open to allow the PVC segment to be placed into the bag. When constructing the microcosms, the PVC segment was placed on top of the soil, and a knife was used to cut through the leaf material and soil before the PVC was gently pressed into the soil. In rocky soils a metal probe was used to find a viable location for the microcosm before placement. The soil-filled core was then lifted using a wide spatula and placed inside the organdy bag. At this point 15 *I. scapularis* nymphs or engorged larvae were added to the core and the bag was fastened shut with a cable tie. Finally, the sealed core containing the ticks, soil, and leaf litter was placed back into the original depression so the edges of the PVC segment were even with the soil surface (Fig. 2.1).

Tick collection and deployment

Nymphal and larval *I. scapularis* were collected from the grounds of the Cary Institute of Ecosystem Studies (41° 47'5.13" N; 73° 44'0.83" W) in the summers (June – August) of 2013 and 2015. Larvae were fed to repletion on lab-raised *Peromyscus leucopus* obtained from the University of South Carolina's *Peromyscus* Genetic Stock Center. All animal handling procedures were approved by a joint IACUC protocol (#2013-0015) between the Cary Institute of Ecosystems Studies and Cornell University. Nymphs and engorged larvae were stored in humidified vials for no longer than three weeks before placement in microcosms. Microcosms used to explore the extraction rates of engorged larvae and actively questing nymphs were placed in the field for 96 hours allowing ticks to locate refugia. To test recovery methods for nymphs in diapause microcosms containing 15 engorged larvae were placed in the field in late August in both 2013 and 2015. They were left in the field for three months and collected in mid-December allowing the engorged larvae to molt into nymphs and engage in behavioral diapause under

natural conditions (Yuval & Spielman 1990). The field site used in 2013 was located in Ithaca NY (42° 28' 4.06" N; 76° 25' 34.21" W), while the 2015 field site was located in Millbrook NY (41° 48' 9.92" N; 73° 44' 30.18" W). Both field sites were located within hardwood forests.

Escape rates and extraction methods

Upon collection, microcosms were tested for recovery methods on *I. scapularis* in three physiological states: engorged larvae, actively questing nymphs, and nymphs in diapause. All microcosms were kept at 21° C for 48 hours prior to processing. Three recovery methods were examined, hand-sorting for 2 hours, hand sorting for 0.5 hours followed by 72 hours in a Berlese funnel (BioQuip collapsible funnel #2832), and 72 hours in a Berlese funnel with no hand sorting. Hand sorting was performed in a large white tub with a tape-lined top edge to prevent ticks from escaping. First the microcosms were cut open and ticks crawling on the inside of the organdy bag were collected, then the soil plug was removed from within the PVC segment. Once inspected, the bag and PVC were set aside and the litter was hand sorted for 10 minutes. This allowed ticks to crawl onto the skin where they could easily be collected. Once the leaf litter was sorted the soil plug was gently broken apart. When recovering ticks without a Berlese funnel the soil was sorted in small volumes in the tray searching for movement while periodically checking the sides for climbing ticks. When a Berlese funnel was used the soil and leaf litter were homogenized and uniformly distributed within the funnel which was placed under a 25-watt bulb. The microcosms that were not hand sorted were removed from the organdy bag and placed directly in a Berlese funnel without homogenization. All samples extracted via Berlese funnel were sorted under a dissecting microscope. All work hours were recorded to provide information regarding the comparative efficiency of these three methods. An additional 30 of the microcosms

containing actively questing *I. scapularis* nymphs were placed in sealed plastic bags for two weeks at 21 °C. The bags were checked daily to determine escape rates.

Statistical Methods

Three ANOVA models were constructed to explore the effect of extraction method on the percent of active nymphs, nymphs in diapause, and engorged larvae recovered from the microcosms. Collection method was included as a factor. A post-hoc Tukey-test was used to determine whether the extraction methods differed from one another. An additional ANOVA model was used to explore differences in work hours between the three methods. All analyses were run in the statistical program 'R' version 3.3.1 (R Core Team 2016).

Results

The overall escape rate from the microcosms was < 1% with only 3 out of 450 nymphs escaping during the two weeks in the laboratory. In all cases, extraction method had a significant impact on tick recovery. For engorged larvae, the hand-sorting method recovered an average of 24.5% (± 3.6) whereas 97.8% (± 1.1) were recovered using a combination of hand sorting and Berlese extraction, and 66.7% (± 5.5) were recovered using Berlese extraction alone ($F = 97.27$; $df = 2, 41$; $P < 0.001$). In the microcosms containing nymphs in diapause 64.6% (± 3.1) were recovered using hand-sorting, whereas 76.3% (± 4.1) were recovered using a combination of hand-sorting and Berlese extraction, versus 43.1% (± 3.3) recovered using the Berlese extraction alone ($F = 22.87$; $df = 2, 46$; $P < 0.001$). In the microcosms containing actively questing nymphs 99.3% (± 0.5) were recovered using hand-sorting, 99.0% (± 0.7) were recovered using a combination of hand-sorting and Berlese extraction, and 90.7% (± 2.6) were recovered using the Berlese extraction alone ($F = 9.79$; $df = 2, 57$; $P < 0.001$) (Fig. 2.2).

Overall the mean number of hours spent recovering ticks per core using hand sorting alone was 2.15 (± 0.01) hours versus 0.89 (± 0.02) hours for a combination of hand sorting and Berlese extraction, whereas Berlese extraction alone took 0.40 (± 0.01) hours (Table 2.1). The work hours include setup and break down times, as well as time spent sorting the Berlese samples under a dissecting microscope. Sorting method had a significant effect on work hours ($F = 4701$; $df = 2, 150$; $P < 0.001$).

Discussion

These results suggest that microcosms are a valid technique to assess *I. scapularis* survival under field conditions, but recovery methods must be selected carefully and are dependent on the life stage targeted. The low escape rate of < 1 % from the microcosms occurred from a single microcosm which may indicate faulty construction of the organdy bag. The recovery rate of nymphs in diapause was lower overall because those microcosms were in the field for three months, during which time natural mortality likely occurred. In contrast, the other cores were in the field for three days, thereby limiting mortality. Both hand-sorting and a combination of hand-sorting and Berlese extraction were effective methods for the recovery of active nymphs and those in diapause. Hand-sorting was the least effective method for the recovery of engorged larvae, likely due to the difficulty of differentiating them from the surrounding soil. Berlese extraction alone was not an effective method for recovery of any life stage. This is likely because ticks burrowed into the soil core and were unable to push through the unbroken core to fall into the ethanol at the bottom of the funnel. Additionally, active nymphs tended to climb into the organdy bag and may have become trapped when not picked out by hand prior to placement in the Berlese funnel. A combination of hand-sorting and Berlese extraction was most effective for the recovery of all physiological states tested, and on average

was 58.7% more time-efficient than hand-sorting, which was the next most effective recovery method. Engorged larvae and nymphs were targeted as they are the smallest life stages that could be contained within the microcosms, since flat larvae were able to escape due to their small size. The recovery methods tested here should therefore be viable for other larger *I. scapularis* life stages and physiological states.

Previous studies that have compared data collected from microcosm experiments to those collected using other techniques show conflicting results (Ginsberg et al. 2004). Comparisons between different methods for examining tick survival and density are important, as methods differ in their confounding factors (Burtis et al. 2016b). Microcosm data cannot assess the effects of host density and diversity or tick behavior on tick densities. These effects can be examined with tick dragging techniques (Schulze & Jordan 2005), or by quantifying small mammal body burdens (Levin & Fish 1998). In turn, these methods provide reliable information regarding *I. scapularis* feeding success (Schmidt et al. 1999, Ostfeld et al. 2006) and entomological risk (Guerra et al. 2002, Diuk-Wasser et al. 2006), but are not the most effective way to measure the direct effect of site-specific factors on *I. scapularis* survival. When using microcosms ticks are placed in the field, thereby eliminating confounding factors related to natural dispersion and host density. Data collected from microcosm experiments allow researchers to focus on the direct effect of site-specific factors, including soil characteristics and forest cover, on tick survival (Bertrand & Wilson 1996, Brunner et al. 2012, Burtis et al. 2016a).

Microcosms are an effective method for the deployment and recovery of *I. scapularis* under field conditions, and the described approach will hopefully encourage more uniform methods allowing for comparisons between microcosm studies. This microcosm method allows ticks to access natural refugium in soil and leaf litter, which has been shown to have a strong

impact on tick survival (Bertrand & Wilson 1996). Despite this importance, the direct effect of refugium availability in differing habitats on tick survival has received little experimental attention (Bertrand & Wilson 1997, Ogden et al. 2006), due in part to the difficulty involved in deploying and recovering ticks from the field under natural conditions. These microcosms provide a suitable proxy for the natural environment of *I. scapularis*, while allowing ticks to be reliably recovered at the end of the experiment.

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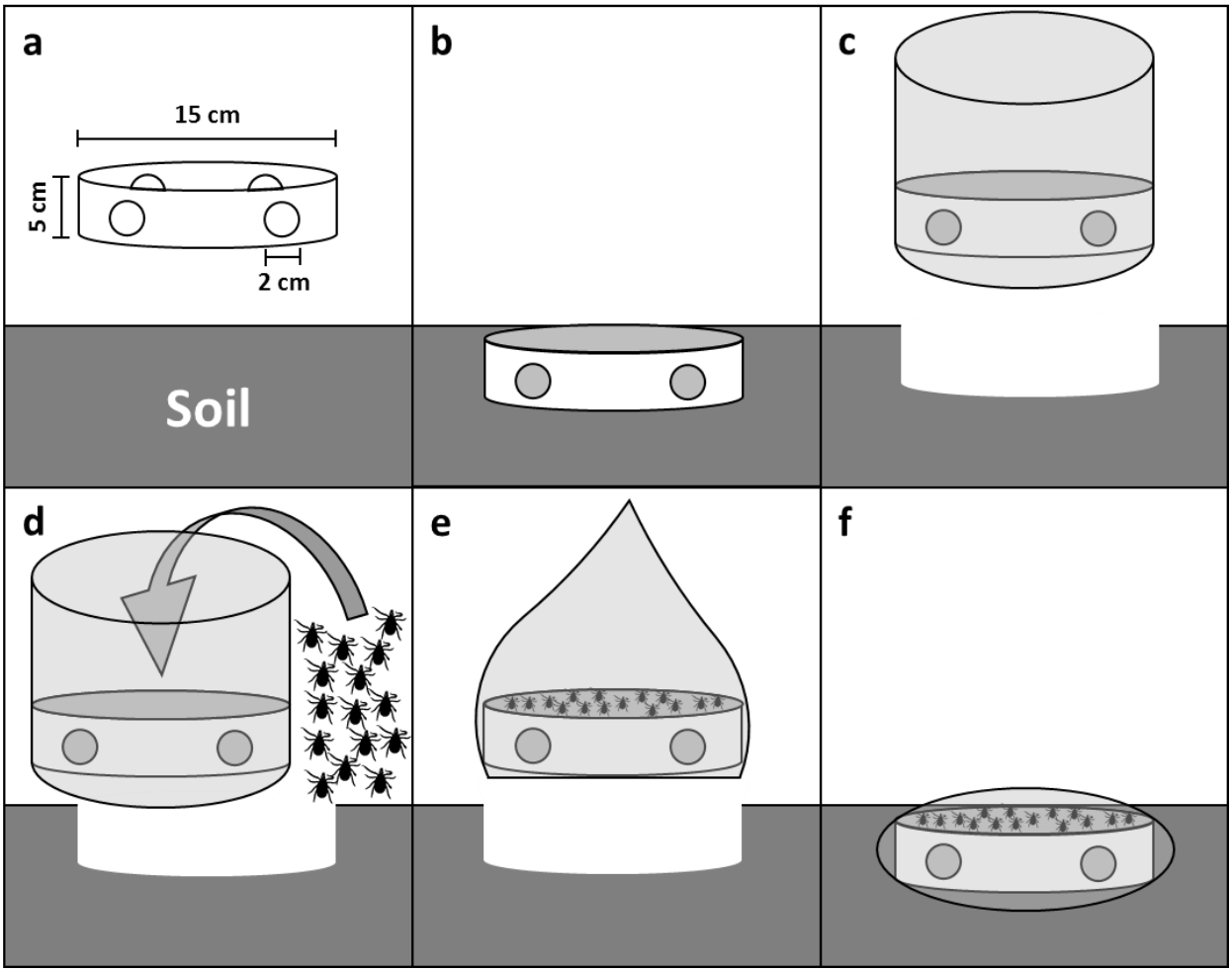

Figure 2.1: Diagram detailing the construction of a microcosm broken into six steps (a – f). The dimensions of the PVC segment are shown above (a). The segment is pushed into the soil creating a soil plug (b), and then is placed into an organdy bag (c). Ticks are then added into the microcosm using a fine brush (d), the bag is sealed (e), and placed back into the original divot (f).

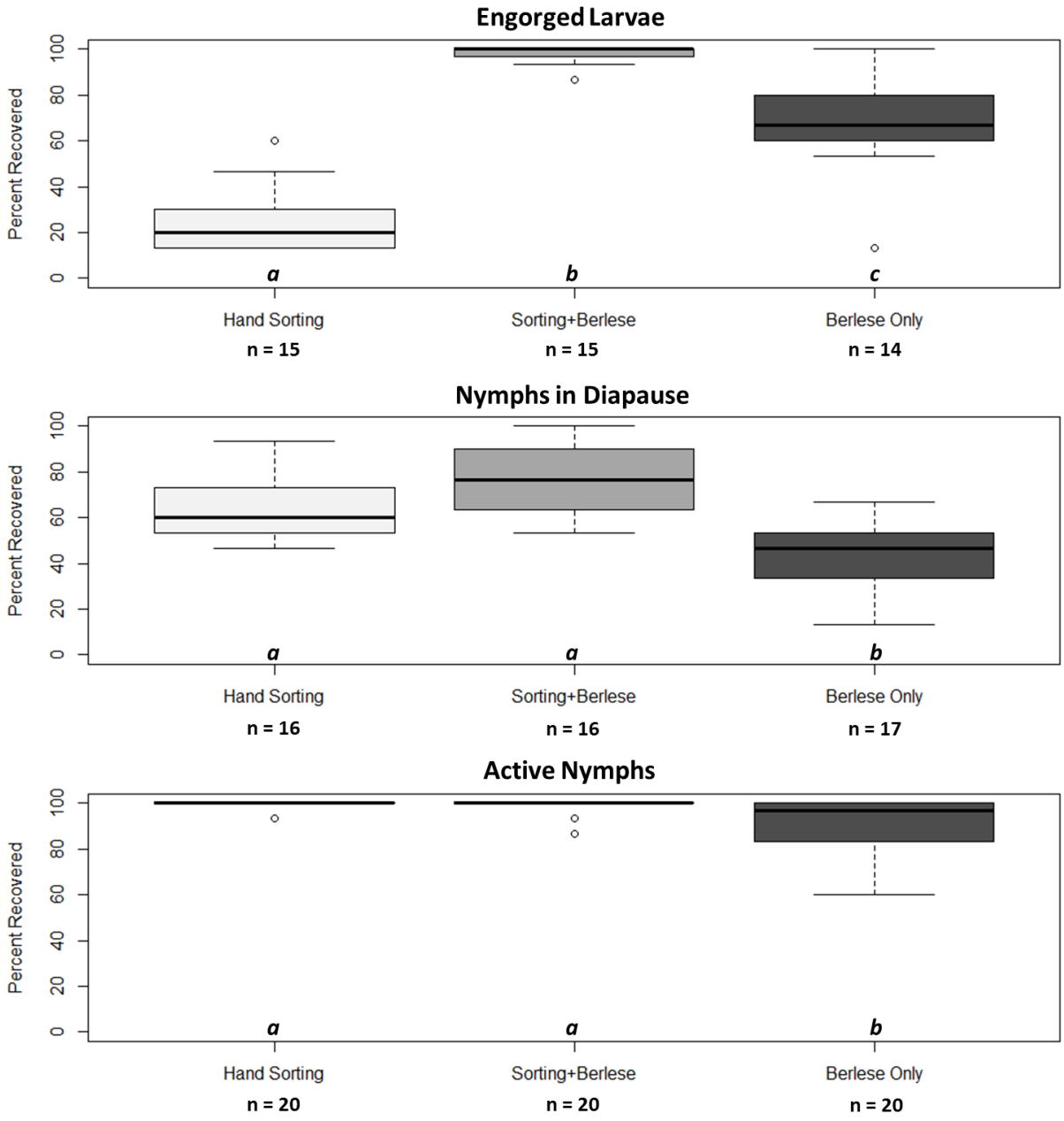

Figure 2.2: Boxplots show the percentage of ticks from the three different physiological states (engorged larvae / nymphs in diapause / actively questing nymphs) recovered using the three methods tested (Hand Sorting / Sorting+Berlese / Berlese). Boxes with different letters differ significantly from one another ($P < 0.05$) according to post-hoc Tukey tests. The numbers below boxes represent sample sizes.

Table 2.1: The mean (± SE) number of work hours per sample processed for each physiological state using the three recovery methods that were tested.

Physiological State	*Hand Sorting*	*Sorting + Berlese*	*Berlese Only*
Engorged Larvae	2.17 (±) 0.02	0.85 (±) 0.03	0.38 (±) 0.02
Diapause Nymph	2.15 (±) 0.01	0.92 (±) 0.03	0.42 (±) 0.03
Active Nymph	2.13 (±) 0.01	0.93 (±) 0.03	0.39 (±) 0.02

CHAPTER 3

THE RELATIONSHIP BETWEEN SOIL ARTHROPODS AND THE OVERWINTER SURIVIAL OF *IXODES SCAPULARIS* (ACARI: IXODIDAE) UNDER MANIPULATED SNOW COVER[3]

Abstract

We explored the relationship between the diversity and abundance of the soil arthropod predator community and the overwinter survival of engorged larval *Ixodes scapularis* Say under variable snow cover in a hardwood forest. We reduced the snow cover over 30 soil core field microcosms simulating predicted changes in snow pack in the northeastern United States. An additional 29 microcosms were used as references with no snow pack manipulation. Each microcosm contained 15 engorged larval *I. scapularis*. We expected lower soil temperature without insulating snow cover to reduce tick survival. However, we observed that reduced snow cover had no effect, with 44.2% and 44.7% overwintering successfully in the reference and snow removal plots, respectively. Increasing taxonomic family richness of arthropod predators, and the total number of large (> 1 mm) arthropod predators significantly reduced the overwinter survivorship of *I. scapularis* within the microcosms. Small (< 1 mm) arthropod predator abundance had no effect. Our results suggest that forests with complex natural arthropod predator communities show reduced tick survival.

[3] Burtis JC, Ostfeld RS, Yavitt JB, and Fahey TJ. 2016. The relationship between soil arthropods and the overwinter survival of *Ixodes scapularis* (Acari: Ixodidae) under manipulated snow cover. Journal of Medical Entomology. 53(1): 225-229. https://doi.org/10.1093/jme/tjv151.

Introduction

Ixodes scapularis (blacklegged tick) Say is the primary vector for many widespread zoonotic diseases in the United States, including Lyme disease, Anaplasmosis, and Babesiosis. This important disease vector spends >95% of its two-year life cycle on or in the soil, but the impact of the soil environment on its survival is understudied, particularly over the winter. Most research regarding *I. scapularis* has focused on interactions with hosts (Ostfeld et al. 2001, Keesing et al. 2009, Kilpatrick et al. 2014), or during their summer/fall activity peaks (Stafford 1994, Berger et al. 2014). With few exceptions (Lindsay et al. 1995, Brunner et al. 2012), factors impacting their overwinter survival in the field have been neglected (Ostfeld & Brunner 2015). Additionally, arthropod predators are known to eat *I. scapularis* in laboratory settings (Samish & Alexeev 2001, Samish et al. 2004), but the impact of arthropod predators, many of which are widespread generalists (Scheu 2001, Digel et al. 2014), has received little investigation.

We explored the impact of snow cover and the composition of the soil arthropod community on the overwinter survival of engorged larval *I. scapularis* as they molted into nymphs in the field. Larvae emerge and feed in late summer, molt into nymphs in the autumn, and then emerge to feed in the late spring the following year (Ostfeld et al. 1996). Laboratory trials have shown that engorged larval *I. scapularis* survival is greatly reduced by temperatures below -10 °C (Burks et al. 1996, Vandyk et al. 1996), but the time scale of these trials was hours rather than weeks or months. Moreover, laboratory conditions often prevent ticks from behavioral adaptations that are available in the field. Snow cover can insulate the soil, and may protect ticks from cold as it does with many other soil-dwelling arthropods (Templer et al. 2012). Snowpack is predicted to decrease in the northeastern United States, with reduced early winter

snowfall (Hayhoe et al. 2008, Campbell et al. 2010), possibly decreasing soil temperatures throughout the winter (Hardy et al. 2001).

Snow cover removal affects soil arthropod communities (Templer et al. 2012), and recent research suggests that changes in soil food web structure can cascade to influence blacklegged tick populations (Coyle et al. 2013, Burtis et al. 2014). Interactions between snow cover manipulation and soil arthropod communities may affect the survivorship of blacklegged ticks. We tested the impact of reduced snow cover, simulating predicted conditions in the northeastern United States, on the overwinter survival of *I. scapularis*, and examined the role of arthropod predator richness and abundance on overwinter survival under reduced snow cover.

Methods

Collecting and Rearing of I. scapularis

Larval *I. scapularis* were collected at the Cary Institute of Ecosystem Studies in Millbrook, New York (41°47'5.13"N; 73°44'0.83"W) in July and August (2013) using a dragging method. Larvae were fed on 18 wild-type *Peromyscus leucopus* from the University of South Carolina's *Peromyscus* Genetic Stock Center. The engorged larvae were placed in humidified vials and stored at 5 °C until they were placed in the field in September 2013. Engorged larvae from individual mice were mixed randomly before placement into microcosms.

Field Installation

Our field installation was located in a northern hardwood forest near Ithaca, NY (42°28'4.06"N; 76°25'34.21"W). The 20 m by 10 m installation contained 63 soil core microcosms. Each microcosm was wrapped in fine mesh cloth and contained within a PVC pipe (10 cm diameter by 5 cm deep) which enclosed soil and leaf material from the site, as well as the associated arthropod community. Additional information regarding microcosm construction is

available in Brunner et al. 2012. Microcosms were clustered into a snow removal group (30 cores), and a reference group (29 cores) where snow cover was not manipulated. Two TidbiT HOBO temperature data loggers, in larvae-free microcosms, were placed in each group (four total). Data loggers were positioned directly under the leaf litter and recorded temperatures every four hours. All non-data logger microcosms contained 15 engorged *I. scapularis* larvae.

Snow was removed from half the plot between 12 November 2013 and 21 January 2014 to simulate predicted snowfall pattern changes in the northeastern United States (Hayhoe et al. 2008, Campbell et al. 2010). All snow was removed except for the first centimeter, which was packed down to minimize leaf litter disturbance. Snow depth was recorded twice a week at 8 points on each plot. Air temperature was recorded at the Northeastern Regional Climate Center, located 4.5 km from our Ithaca field site.

Collection of I. scapularis and Other Arthropods

I. scapularis and other arthropods were collected from the microcosms between 3 July and 28 July 2014. Soil and leaf materials from each microcosm were hand sorted for 2 hours (Fig. 3.1). Afterward all materials were placed in Berlese funnels for seven days to collect additional nymphs and arthropods. All arthropods were sorted to taxonomic order. Arthropod predators in the class Chilopoda, and the orders Araneae, Mesostigmata, Pseudoscorpionida, and Trombidiformes were sorted to taxonomic family (Dindal 1990, Ubick & Cushing 2005, Krantz & Walter 2009).

Statistical Analyses

We used three generalized additive models to examine differences in daily mean soil temperatures between the snow removal and reference plots; 1) examined temperature differences before the snow removal period, 2) differences during the snow removal period, and

3) differences after the snow removal period. We used a cubed-root transformation on the temperature data and all three models included ‘snow removal’ as a factor, and ‘date’ as a smoothing term. We explored the impact of snow removal on *I. scapularis* survival with a Student t-test.

We ran three mixed-effects linear models examining the relationship between the litter community and *I. scapularis* overwinter survival including the effects of; predator family diversity, large (> 1 mm) arthropod predator (Araneae / Chilopoda) abundance, and small (< 1 mm) arthropod predator (Mesostigmata / Pseudoscorpionida / Trombidiformes) abundance. A fourth model examined the effect of predator family richness on large arthropod predator abundance. Collection date was included as a random effect, accounting for temporal variation in arthropod predator populations. P-values were corrected for multiple comparisons using a false-discovery rate method (Benjamini and Hochberg 1995). Analyses were run in the statistical program ‘R’ (R Core Team 2014).

Results

During the snow removal period snow depth averaged 4.33 cm (± 0.38) on the reference plot. There was no significant difference in soil temperatures between the snow removal and reference plots prior to the snow removal ($F = 0.40$; $df = 1, 78$; $P = 0.529$), but mean daily soil temperatures were 0.62 °C lower in the snow removal compared to the reference plot during the snow removal period ($F = 19.49$; $df = 1, 113$; $P < 0.001$). After the snow removal period there was a small, but significant difference in soil temperature ($F = 5.45$; $df = 1, 81$; $P = 0.022$). The lowest air temperature recorded was -19.75 °C, while the lowest soil temperature was -4.23 °C (Fig. 3.2). *I. scapularis* survival (percentage of nymphs recovered) was not impacted by snow

removal ($t = 0.071$; $df = 53$; $P = 0.944$), with 44.2% (± 4.96) and 44.7% (± 3.91) surviving in the snow removal and reference plots, respectively.

Arthropods from 17 taxonomic orders were found in our microcosms, and 13 arthropod predator families were identified (Tables 3.1 and 3.2). The dominant large arthropod predators were in the family Lithobiidae (Chilopoda). The dominant small arthropod predator family was Parasitidae (Mesostigmata). Arthropod predator family richness ($t = -3.59$, $df = 57$, $P < 0.001$) (Fig. 3.3A), and large arthropod predator abundance ($t = -3.82$, $df = 57$, $P < 0.001$) (Fig. 3.3C) both had a significant negative effect on *I. scapularis* survival. There was no relationship between small arthropod predator abundance and *I. scapularis* survival ($t = -1.23$, $df = 57$, $P = 0.226$) (Fig. 3.3B). There was also a positive relationship between predator family richness and large arthropod predator abundance ($t = 5.42$, $df = 57$, $P < 0.001$) (Table 3.3). Snow removal treatment had no effect, and was not included in these analyses ($\Delta AIC<2$).

Discussion

Snow removal had no impact on the overwinter survival of *I. scapularis* in our microcosms. Despite air temperatures below -19 °C, temperatures under the leaf litter, where *I. scapularis* overwinters (Yuval & Spielman 1990, Daniels et al. 1996), never dropped below -4.23 °C. Laboratory trials suggest survival of engorged larval *I. scapularis* is not strongly reduced until temperatures reach -10.83 °C (Vandyk et al. 1996). Our snow removal treatment had minimal impact on the soil temperature, partly because the snow cover during the removal period was intermittent (Fig. 3.2). It seems unlikely that predicted changes to winter soil temperature as a result of reduced snow cover in regions with intermittent early winter snow cover will have a strong direct effect on the overwinter survival of *I. scapularis*. Our results agree with previous field observations of the effect of temperature on *I. scapularis* overwinter

survival (Lindsay et al. 1995, Brunner et al. 2012). It is possible that prolonged exposure to cold in areas with a permanent early winter snow pack, where snow removal has a stronger effect on soil temperature (Decker et al. 2003, Templer et al. 2012), would impact *I. scapularis* overwinter survival. More study is needed in these regions.

In contrast, soil arthropod predator community richness had a significant negative effect on nymphal survival (Fig. 3.3A), unrelated to snow removal treatment. The density of large arthropod predators, predominantly centipedes in the family Lithobiidae, had a negative relationship with *I. scapularis* survival (Fig. 3.3C), while no such trend existed for small arthropod predators (Fig. 3.3B). It is possible that large arthropod predators targeted *I. scapularis* in our microcosms, while predatory mites were too small to attack *I. scapularis*. Additionally, we observed that as the arthropod predator communities grew more complex, as measured by family richness, more large arthropod predators were present. This may indicate that as soil communities grow more complex they can support more large arthropod predators, as previously hypothesized (Chen & Wise 1999, Kalinkat et al. 2013).

The density and richness of the soil arthropod communities in our microcosms were lower than those observed in other studies (Burtis et al. 2014). The impact of some larger arthropods, particularly spiders, may actually be greater than observed in our microcosms. Additionally, our lack of knowledge regarding interactions between *I. scapularis* and arthropod predatory taxa make it difficult to directly link the decline in *I. scapularis* survival to the predators in the cores. Our data represent a snapshot of the arthropod community as we could not detect inactive or dead arthropods. Thus, predators not active in July were unaccounted for, again leading to an underestimation of their potential impact.

The impact of the soil food web on *I. scapularis* survival has received little study, and our data suggest that it warrants further evaluation. It is increasingly evident that *I. scapularis* density can be impacted by many factors within the soil ecosystem (Coyle et al. 2013, Burtis et al. 2014). This study focused on one portion of the *I. scapularis* lifecycle and is limited in scale, but indicates that soil-dwelling arthropod predators may affect *I. scapularis* survival. Further studies may help clarify which factors affect the localized spatial distribution of this important disease vector.

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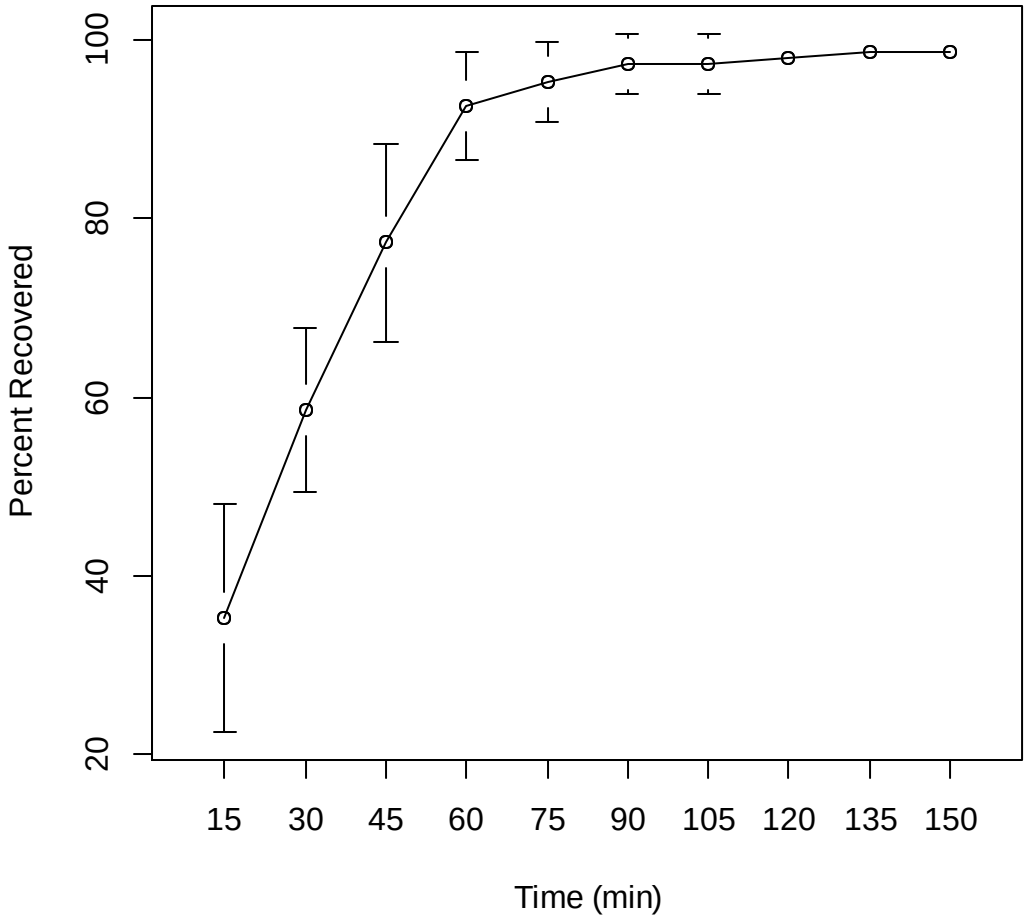

Fig. 3.1: The recovery rate of nymphs from 10 soil core microcosms that contained 15 nymphal *I. scapularis* each. These microcosms were left out in the field for 1 week to allow nymphs to disperse. Soil core microcosms were then collected and hand sorted in the laboratory for 2.5 hours each. The number of nymphs collected was recorded every 15 minutes. After 2 hours of hand sorting 98% of the nymphs were recovered.

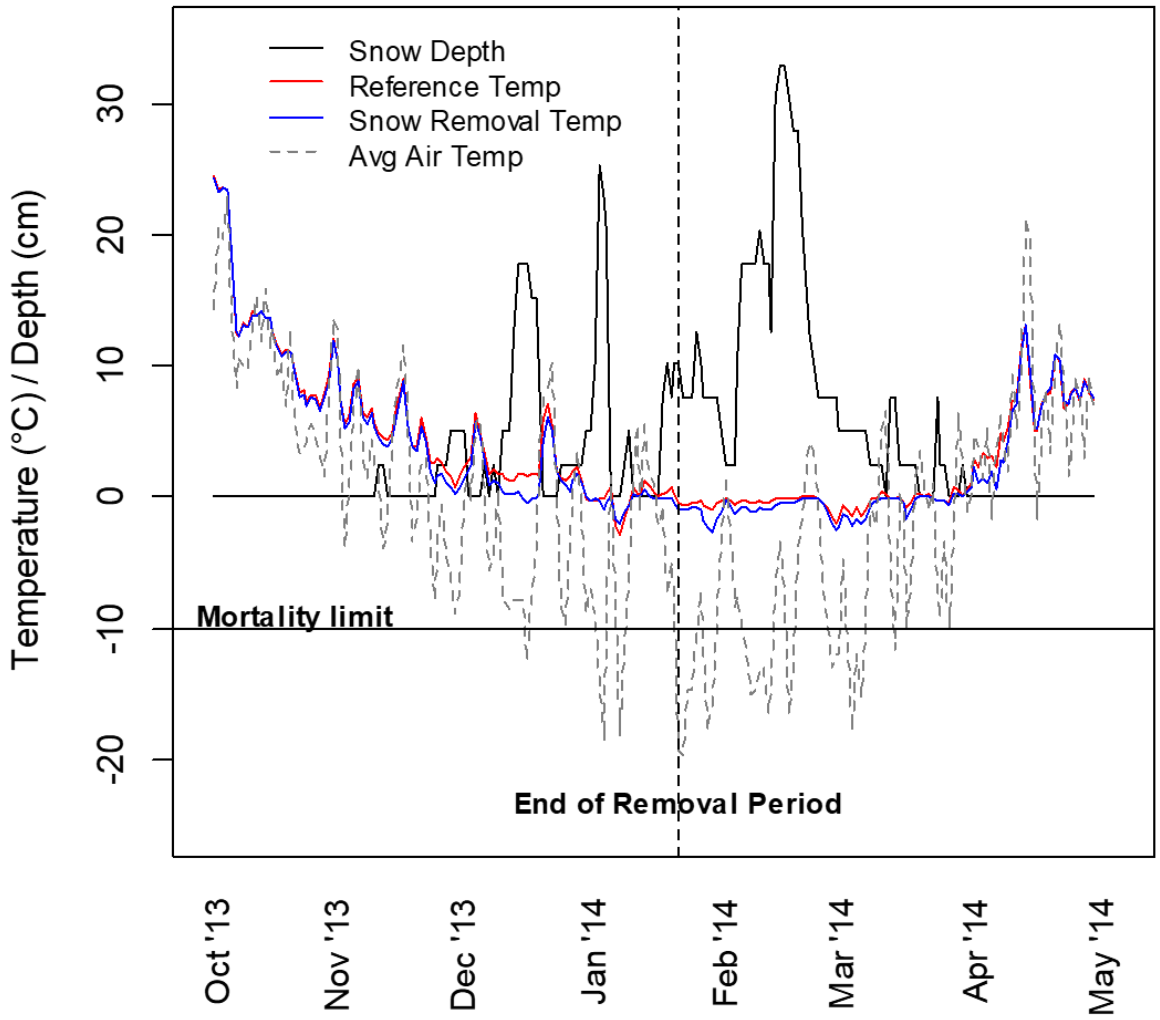

Figure 3.2: Mean daily temperatures recorded at the litter-soil interface from the data loggers in the snow removal (blue), and reference (red) plots, along with the average air temperature (dashed-grey), and snow depth on the reference plot (black). The vertical dashed line signifies the end of the snow removal period. The horizontal solid line represents the temperature at which *I. scapularis* mortality begins to increase substantially (Vandyk et al. 1996). The difference in mean snow depth between the plots after February 4th was 0.12 cm.

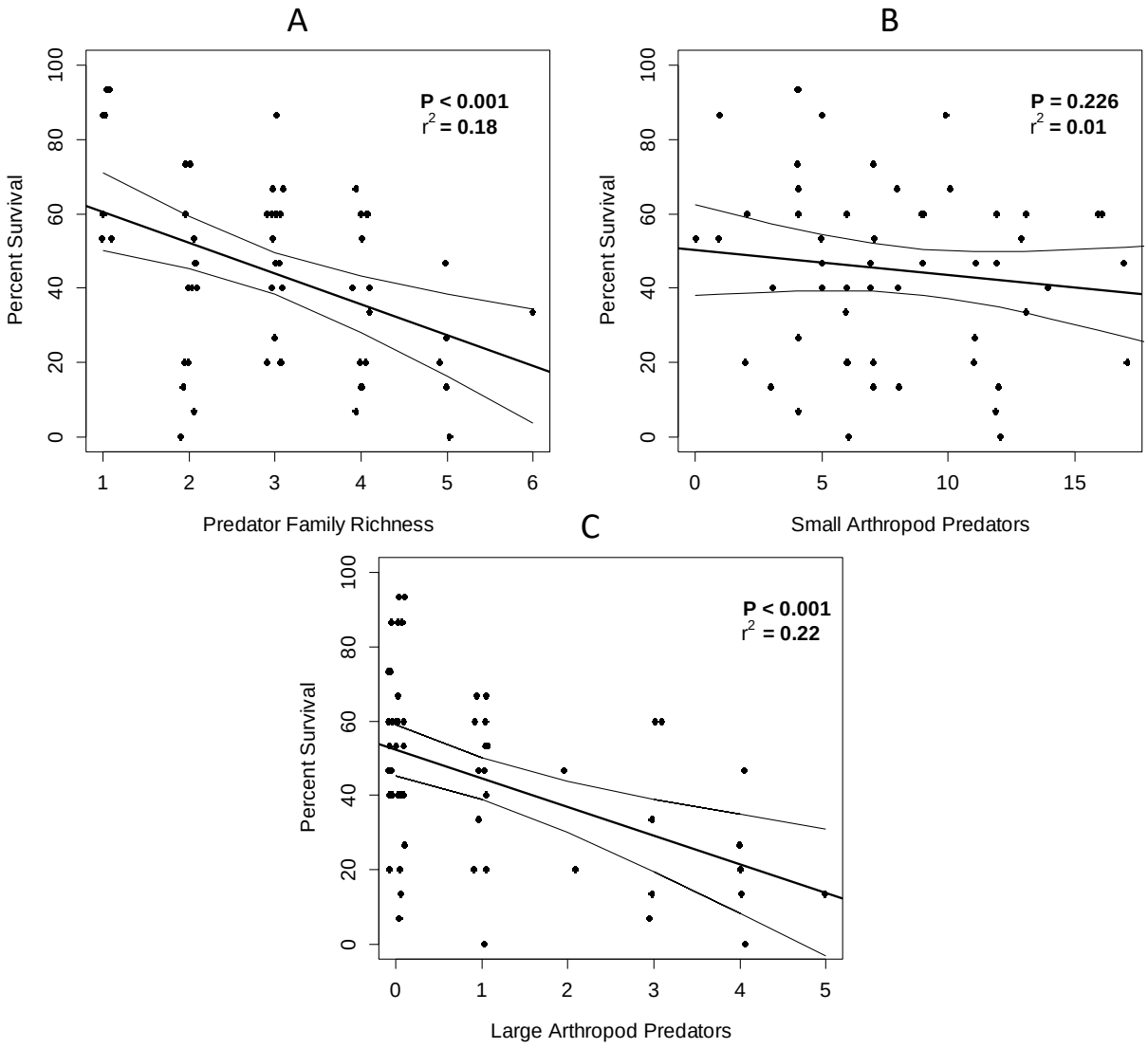

Figure 3.3: The percentage of engorged larval *I. scapularis* that overwintered successfully plotted against; (A) arthropod predator family richness, (B) small arthropod predator abundance, and (C) large arthropod predator abundance, with best fit lines and 95% confidence intervals. Noise has been added to the data to make overlapping values visible.

Table 3.1: Arthropod order average counts (±) 1 SE in the snow removal (n = 30) and reference (n = 29) microcosms. Orders that contained large predators (> 1 mm) are bolded.

Taxonomic Order	Snow Removal	Reference
Araneae	**0.06 (±) 0.06**	**0.04 (±) 0.04**
Coleoptera	0.94 (±) 0.17	0.61 (±) 0.19
Diptera	0.65 (±) 0.19	0.36 (±) 0.15
Entomobryomorpha	2.29 (±) 1.23	2.04 (±) 0.60
Geophilomorpha	**0.00 (±) 0.00**	**0.04 (±) 0.04**
Hymenoptera	0.39 (±) 0.13	0.25 (±) 0.13
Isopoda	0.00 (±) 0.00	0.61 (±) 0.18
Julida	0.97 (±) 0.27	1.25 (±) 0.27
Lepidoptera (larvae)	0.19 (±) 0.09	0.25 (±) 0.08
Lithobiomorpha	**0.94 (±) 0.28**	**1.54 (±) 0.28**
Mesostigmata	34.0 (±) 2.65	29.8 (±) 3.10
Poduromorpha	1.35 (±) 1.05	20.9 (±) 10.3
Polydesmida	0.00 (±) 0.00	0.36 (±) 0.21
Pseudoscorpionida	0.23 (±) 0.09	0.21 (±) 0.09
Sarcoptiformes	83.4 (±) 6.56	103.4 (±) 9.54
Symphypleona	0.06 (±) 0.06	0.04 (±) 0.04
Trombidiformes	0.16 (±) 0.07	0.04 (±) 0.04
Total	125.6 (±) 8.84	161.7 (±) 15.1

Table 3.2: The total number of arthropod predators collected from the microcosms organized by taxonomic family within order.

Arthropod Families	Total Count
Araneae	
Clubionidae	1
Dictynidae	2
Geophilomorpha	
Geophilidae	2
Lithobiomorpha	
Lithobiidae	52
Mesostigmata	
Eviphididae	8
Pachylaelapidae	1
Parasitidae	232
Rhodacaridae	29
Ologamasidae	208
Veigaiidae	10
Pseudoscorpionida	
Chthoniidae	1
Neobisiidae	11
Trombidiformes	
Trombidiidae	6
Total	*563*

Table 3.3. Results of three linear mixed effects models of factors affecting overwinter *I. scapularis* survival including; (A) arthropod predator family richness, (B) large (> 1 mm) arthropod predator abundance, and (C) small (< 1 mm) arthropod predator abundance. Model D shows the effect of arthropod predator family richness on large arthropod predator abundance. Collection date was included in each model as a random effect. All p-values were corrected using Benjamini & Hochberg's false discovery rate method (Benjamini and Hochberg 1995).

Model A					
Factor	***Estimate***	***SE***	***t-value***	***df***	***p-value***
Family Richness	10.33	0.35	-3.59	1, 57	<0.001*
Model B					
Factor	***Estimate***	***SE***	***t-value***	***Df***	***p-value***
Large Predators	7.83	0.52	-3.82	1, 57	<0.001 *
Model C					
Factor	***Estimate***	***SE***	***t-value***	***df***	***p-value***
Small Predators	-0.11	0.09	-1.23	1, 57	0.2257
Model D					
Factor	***Estimate***	***SE***	***t-value***	***df***	***p-value***
Family Richness	0.66	0.12	5.42	1, 57	<0.001*

CHAPTER 4

INTERACTIONS BETWEEN SOIL-DWELLING ARTHROPOD PREDATORS AND *IXODES SCAPULARIS* UNDER LABORATORY AND FIELD CONDITIONS[4]

Abstract

The primary vector for Lyme disease and several other medically significant tick-borne diseases, *Ixodes scapularis* (blacklegged tick), spends over 95% of its two-year lifecycle exposed to the soil environment. However, the effect of soil-dwelling arthropod predator populations on *I. scapularis* survival either in the laboratory, or under field conditions is unclear. We collected soil-dwelling obligate arthropod predators from the field, representing 13 taxonomic families, and investigated whether they would target two *I. scapularis* life stages (nymph / engorged larva) under laboratory conditions. After 48 hours, 30.6% of the predator species targeted *I. scapularis* nymphs, while 41.3% targeted engorged larvae. One spider species, *Schizocosa ocreata*, targeted both *I. scapularis* life stages reliably under laboratory conditions. We used this species to test whether an arthropod predator would affect *I. scapularis* survival in field microcosms which contained alternative prey items and a complex soil environment. We also investigated the direct and indirect effects of surface litter on *I. scapularis* survival by removing the leaf litter layer from half of the microcosms. Predator addition reduced the number of nymphs recovered from the microcosms after 21 days by 32.9%. Litter removal also had a negative effect on tick survival, and interacted weakly with the addition of a predator further decreasing tick survival.

[4] Burtis JC and Pflueger C. 2017. Interactions between soil-dwelling arthropod predators and *Ixodes scapularis* under laboratory and field conditions. Ecosphere. 8(8): e01914. https://doi.org/10.1002/ecs2.1914.

Additionally, we found that when leaf litter was removed *I. scapularis* survival was positively correlated with the organic matter content of the soil within the microcosms. Naturally occurring arthropod predators may play an important role in regulating population dynamics of *I. scapularis*, and show potential as biological control agents for use in integrated pest management protocols.

Introduction

The blacklegged tick (*Ixodes scapularis* Say) is the primary vector for a variety of common tick-borne diseases in North America (Nelson et al. 2015) and spends the majority of its two-year lifespan exposed to the soil environment. Much *I. scapularis* research has focused on factors affecting questing behavior and their interactions with vertebrate hosts (Ginsberg & Zhioua 1999, Keesing et al. 2009, Kilpatrick et al. 2014). During its life cycle *I. scapularis* spends approximately two weeks interacting directly with hosts, while the remaining time is spent in the upper layers of the soil either in diapause (Lindsay et al. 1998, Ogden et al. 2004), or questing on leaf litter and low lying vegetation searching for hosts (Schulze & Jordan 1995, Williams & Ward 2010). Despite the disproportionate amount of time *I. scapularis* spends in the soil limited experimental investigation has been conducted on the factors affecting tick mortality during this period (Ostfeld & Brunner 2015). Much of the research focusing on this period of their life cycle has employed indirect methods, such as tick dragging or host body burden, both of which can be biased by tick behavior and host density (Ginsberg & Ewing 1989, Schulze et al. 1997, Levin & Fish 1998). Furthermore, many conclusions about soil factors affecting tick population dynamics have been based upon correlative data (Guerra et al. 2002, Brownstein et al. 2003, Diuk-Wasser et al. 2006), thereby making it difficult to ascribe cause and effect. Here we experimentally evaluate the efficacy of a variety of common soil-dwelling arthropods as predators of *I. scapularis* under laboratory conditions, and examine the effect of a common wolf spider, *Schizocosa ocreata* (Hentz), on the survival of questing *I. scapularis* under variable field conditions.

Various soil biota, including earthworms (Burtis et al. 2014), entomopathogenic fungi (Benjamin et al. 2002), and nematodes (Zhioua et al. 1995, Hill 1998) have been shown to affect

I. scapularis survival and population densities. However, arthropod predators represent an understudied group of organisms, a variety of which are known to target *I. scapularis* under laboratory conditions (Samish & Alekseev 2001, Samish 2004). Arthropod predators are often present in high densities in the soil ecosystem (Moulder & Reichle 1972, Lensing et al. 2005) and are also known to exert a strong top-down force on other soil-dwelling arthropods (Clarke & Grant 1968, Lawrence & Wise 2000). Predation of ticks by various arthropods is well-documented, particularly by various species of ant (Formicidae) and predatory beetles (predominantly Carabidae) (Samish & Alekseev 2001), but few studies have focused on common non-insect arthropod predators including centipedes and spiders. These taxa have the potential to be important predators of *I. scapularis* due to their relatively high densities in the temperate forests of eastern North America (Burtis et al. 2014). For example, the common spider *S. ocreata* occurs widely in the forests of the northeastern United States and has been found to target *I. scapularis* under laboratory conditions (Carroll 1995). Whether this species will target *I. scapularis* under field conditions has yet to be tested, nor has the efficacy of other common spiders and centipedes as predators of *I. scapularis* been evaluated.

Tick survival also depends on microhabitat availability (Semtner et al. 1971, Bertrand & Wilson 1997, Shaw et al. 2003, Kerr & Bull 2006). Access to leaf litter has been shown to affect survival (Bertrand & Wilson 1996) and litter removal reduces the density of questing nymphs (Schulze et al. 1995, Stafford et al. 1998). However, previous litter manipulation experiments are likely to have simply removed the existing population of ticks as well as arthropod predators. These results are meaningful from a management perspective as litter removal should reduce human contact with questing ticks (Maupin et al. 1991), but may not provide reliable information regarding the direct effect of the litter environment on *I. scapularis* survival. The direct effect of

litter removal on the survival of *I. scapularis* under field conditions has not been evaluated experimentally, nor has the effect of other soil properties such as soil organic matter content, which increases soil moisture, and in turn stabilizes variation in temperature and relative humidity in the soil environment (Vreeken-Buijs et al. 1998). This microhabitat stabilization may provide *I. scapularis* with valuable refugia in highly organic soils with limited leaf litter cover.

In addition to the direct effect of the physical soil environment on tick survival there is also potential for indirect effects through interactions with soil biota. For example, entomopathogenic fungi are more common on the soil surface than in leaf litter (Schulze & Jordan 1995, Tuininga et al. 2009), and the removal of leaf litter may increase contact rates between *I. scapularis* and these fungi. Predator foraging patterns are also strongly affected by habitat complexity (Uetz 1979, Finke & Denno 2002), with wandering predators being inhibited by complex environments which provide refugia for prey, while ambush predators may find increased hunting success in complex environments due to the availability of concealed areas (Langellotto & Denno 2004). Because of high rates of intraguild predation (Rosenheim et al. 1993, Wise & Chen 1999) the use of obligate arthropod predators as biological control agents has shown mixed results with non-tick target taxa (Riechert & Lockley 1980, Hodge 1999), but they have been shown to consume large numbers of target prey when applied appropriately (Mansour et al. 1980, Mansour & Whitecomb 1986, Spiller 1986, Snyder & Wise 1999).

The present study has three primary objectives; first to evaluate whether a suite of common arthropod predators will target two *I. scapularis* life stages (nymph / engorged larva) under laboratory conditions, second to quantify the direct impact of a known tick predator (*S. ocreata*) on the survival of ticks in field microcosms containing a complex soil environment and alternative prey items, and third to determine the effects, both direct and indirect (via the

predator), of litter removal on tick survival in the field. Improving our understanding of which arthropod predators target *I. scapularis*, and how these predators behave under variable field conditions are the initial steps toward identifying novel biological control agents for this important disease vector.

Methods

Field site descriptions

This study was conducted on the grounds of the Cary Institute of Ecosystem Studies (CIES) in Millbrook NY (41° 47’5.13” N; 73° 44’0.83” W). The field trials were conducted on three forested plots on the property. We named field sites after the CIES structure in closest proximity, 1) Bacon Flats (N 41°46'54.05"; W 73°44'01.64"), 2) Field Lab (41°47'59.17"N; 73°43'55.07"W), and 3) Rearing Facility (N 41°47'25.97"; W 73°45'31.59"). Sites were selected for similar topography, tree cover, and vegetation. All field sites had slopes between 0 - 5%, with rocky Nassau-Cardigan complex soils. The dominant tree species on all three sites were *Acer rubrum*, *A. saccharum*, and *Quercus rubra*. In order to avoid a patchy distribution of microhabitats all field sites were selected to have limited understory vegetation.

Arthropod predator and tick collections

Nymphal *I. scapularis* were collected between June 1st 2015 and June 23rd 2015 and again on the same dates in 2016 on CIES grounds using the tick dragging method (Schulze et al. 1997). Nymphs were stored in humidified vials for less than two weeks before use. The engorged larvae that were used in the laboratory palatability trials were collected as flat larvae on CIES grounds between July 29th 2015 and August 16th 2015. Larvae were immediately fed on lab-raised *Peromyscus leucopus* obtained from the Peromyscus Genetic Stock Center at South Carolina University. *P. leucopus* were inoculated with larvae and kept in wire mesh cages above

a tape-lined tray with a moistened paper towel in the bottom. Trays were checked every 12 hours after inoculation with engorged larvae. All animal handling procedures were approved by a joint agreement between both the CIES and Cornell University Institutional Animal Care and Use Committees (protocol #2013-0015).

Arthropod predators were collected on the grounds of CIES between May 28th 2015 and September 2nd 2015, and again between June 1st 2016 and June 11th 2016. We used a variety of collecting methods including; direct hand collection, sweep netting, and litter sorting. Predators were stored in humidified vials for less than three days before being placed either in the laboratory palatability trials or added to microcosms in the field.

Laboratory palatability trials

Palatability trials involved presenting a single *I. scapularis* nymph or engorged larva to a predator to determine whether the predator would identify the tick as a prey item. Prior to engaging in the palatability trials all predators were fed one or two (for larger predators) flightless *Drosophila melanogaster* (Obtained from Timberline Fisheries) and left in humidified 20 ml plastic vials for 48 hours. This allowed us to limit the confounding effect of predator field condition by ensuring that all predators were fed a meal prior to contact with the ticks. After 48 hours, we added either a single *I. scapularis* nymph, or engorged larva into the humidified vial with the predator. Predation events were recorded every 12 hours over the next two days. Once the palatability trial was completed predators were placed in 70% ethanol to be identified later. Spiders were identified to species (Ubick & Cushing 2005, Bradley 2012), and centipedes to genus (Weaver 1967, Kevan 1989, Barber 2009).

Field microcosms

A field microcosm experiment was established on the three replicate sites described earlier. We laid out a 121-point randomization grid over a 30 m x 30 m plot on each of the three field sites with each point separated by three meters. Treatments included predator addition and leaf litter removal in a 2x2 factorial design with 40 microcosms on each of the sites. The locations of treatment microcosms were randomly assigned using the grids established on each site. Microcosms were set out in the field for 21 days. We staggered setup and collection by five days starting on June 22nd 2015 and ending on July 17th 2015 to allow time to process the microcosms and avoid storing them for differing amounts of time after collecting them from the field. Two microcosms were destroyed by animals during the study, one from the reference group from the Field Lab site, and one predator addition sample from the Rearing Facility site. These samples were not included in our analyses.

Microcosms were constructed using a PVC segment wrapped in an organdy bag. The PVC segment had a diameter of 10 cm and a depth of 5 cm with a total volume of 392.7 cm^3. Microcosms were constructed by placing the PVC segment on the forest floor, placing the litter inside the segment to the side, and then pounding the PVC into the upper layer of the soil. Leaf litter was then placed on top of the PVC segment containing the soil plug and everything was placed into a fine mesh bag. We then added 15 *I. scapularis* nymphs to each microcosm using a brush. We also added a single *S. ocreata* to the predator addition microcosms, and permanently removed the leaf litter from the litter removal treatment group. Finally, the bags were sealed to prevent *I. scapularis* nymphs and *S. ocreata* from escaping. The fine organdy wrapped around each microcosm allowed weather conditions to affect the microhabitat in the core with minimal interference. Leaf litter was also removed from a 2 m x 2 m area around each litter removal treatment to prevent the surrounding litter from collecting on top of the microcosms over time.

Litter was cleared a week prior to the initiation of the experiment to allow the soil surface contact with the air. Litter treatments were checked biweekly to ensure that leaf litter from the surrounding area was not encroaching on the microcosms.

Six identical microcosms were installed at each site to monitor relative humidity and temperature using HOBO dataloggers (HOBO U23 v2). Dataloggers were set to record at 2 hour intervals. To determine whether the litter removal treatment directly affected the microhabitat within the microcosms leaf litter was removed from half of them, while the litter was left unmanipulated in the other half. Dew points were calculated using the relative humidity and temperature data. Daily variation in dew point was determined as the difference between the peak high and low daily values (referred to as ΔDew hereafter).

Tick recovery and soil measurements

After 21 days in the field the microcosms were collected and returned to the laboratory. Soil and leaf litter from each microcosm were hand sorted for 1 hour to recover *I. scapularis* nymphs and predators and then placed in a Berlese funnel under a 25-watt bulb for 72 hours. This allowed us to collect additional nymphs, as well as any other naturally occurring arthropods present in the microcosms which were alternative prey items. Arthropods were stored in 70% ethanol before being identified to taxonomic class using the available guides (Gibb & Oseto 2006, Krantz & Walter 2009) and classified as either microarthropods (Collembola / Pseudoscorpionida / Acari) or macrofauna (Araneae / Insecta / Chilopoda / Diplopoda). After arthropod extraction, the soil from each microcosm was homogenized, and a subsample was dried at 65 °C for 48 hours and weighed. The subsample was then placed in a muffle furnace at 400 °C for four hours and reweighed to determine loss on ignition as an estimate of soil organic

matter loss (% SOM). Using this method organic matter is burned off leaving only the mineral soil behind (Schulte et al. 1991, Goldin 2008).

Statistical methods

To determine predator preference for different *I. scapularis* life stages (nymph / engorged larva) in our predator palatability trials we ran a binomial mixed effects model. This model included predation events (yes / no) as the dependent variable with *I. scapularis* life stage as the fixed effect. The taxonomic genus of the arthropod predators was included as a random effect in this model to account for differences between genera.

We used a mixed effects linear model to explore the effects of our field microcosm experiment on the survival of *I. scapularis* nymphs over the 21-day trial period. Litter removal, arthropod predator addition, and %SOM were included as fixed effects, while site was included as a random effect. Inclusion of variables and interactions were evaluated using the Akaike information criterion (AIC) scores. Another mixed effects linear model was used to compare the effect of litter removal on daily variation in dew point (ΔDew). For this analysis ΔDew data were log-transformed and site was again included as a random effect. Finally, we ran two mixed effects linear models to compare the effect of litter removal and predator addition on microarthropod and macrofaunal populations; site was included as a random effect and all soil arthropod data were log transformed. Collection date was not included in these models because it did not improve them according to their AIC scores ($\Delta AIC > 2$). All statistical models were run in R version 3.3.1 (R Core Team 2016).

Results

A total of 336 arthropod predators, including 26 different species divided among 13 taxonomic families were collected during the summers of 2015 and 2016 for the tick palatability

trials (Table 4.1). Predators were significantly more likely to target engorged larvae than nymphs ($z = -4.06$; $df = 1, 308$; $P < 0.001$). Overall, predators targeted engorged larvae in 41.3% of the cases while only 30.6% of nymphs were targeted. The species which most commonly attacked both *I. scapularis* life stages was *S. ocreata* which targeted 93.8% of nymphs and 94.7% of engorged larvae. Other species of spider also targeted *I. scapularis* during the palatability trials, notably *Phidippus audax* (Hentz) which targeted nymphs in 57.8% of the cases, while 100% of engorged larvae were eaten. Overall, occurrence of predation events tended to vary widely across predatory taxa. Centipedes rarely targeted either life stage, but occasionally species in the family Lithobiidae fed on *I. scapularis*, most commonly targeting engorged larvae. A detailed list of all predators tested during our laboratory palatability trials, along with the timing and occurrence of predation events, can be found in table 4.2.

In the field microcosms the removal of leaf litter significantly affected microhabitat availability as represented by ΔDew ($F = 9.07$; $df = 1, 164$; $P = 0.003$); there was more daily variation in dew point when the litter was removed (Fig. 4.1). The mean survival rate of *I. scapularis* nymphs after the 21-day period in the reference microcosms, without litter removed or a predator added, was 77.0% (± 3.7). In the litter removal microcosms 70.4% (± 2.9) of the nymphs survived, while 44.4% (± 4.1) survived in the predator addition treatment. In the interaction treatment (predator addition + litter removal) 44.1% (± 5.1) of the nymphs survived (Fig. 4.2). Both the litter removal ($F = 7.58$; $df = 1, 110$; $P = 0.007$) and predator addition ($F = 47.53$; $df = 1, 100$; $P < 0.001$) treatments had significant effects on tick survival. A marginally non-significant interaction between the two treatments ($F = 3.07$; $df = 1, 110$; $P = 0.08$) suggested a weak effect of litter removal on predator foraging (Table 4.3). The % SOM had a significant effect on the survival of nymphs in the microcosms ($F = 12.66$; $df = 1, 110$; $P <$

0.001), and there was a significant interaction between litter removal and %SOM (F = 7.59; df = 1, 110; P = 0.007) with %SOM having a stronger effect in microcosms in the litter removal treatment (Fig. 4.3). Other interactions (% SOM + predator addition / SOM + predator addition + litter removal) did not improve the model according to the AIC scores (ΔAIC < 2), and were not included in our final model (Table 4.4).

The treatments had effects on the density of alternative prey, macrofauna and microarthropods, in the microcosms. For the macrofauna, we recovered an average of 16.55 (± 5.13) individuals from the reference microcosms, 6.10 (± 0.79) from those in the litter removal treatment, and 6.73 (± 0.59) from the predator addition treatment. Both litter removal (F = 47.42; df = 1, 112; P < 0.001) and predator addition (F = 25.64; df = 1, 112; P < 0.001) had a significant effect on macrofauna densities, but the interaction between the two treatments was not significant (F = 0.03; df = 1, 112; P = 0.858) (Fig. 4.4A). For the microarthropods, 329.00 (± 111.32) individuals were recovered on average from the reference microcosms. Only the leaf litter removal treatment significantly affected microarthropod densities (F = 58.13; df = 1, 112; P < 0.001), with an average of 106.57 (± 15.41) individuals recovered. Predator addition had a marginally significant effect on microarthropod densities (F = 4.08; df = 1, 112; P = 0.046) with 217.03 (± 33.83) microarthropods recovered from these microcosms. There was no interaction between predator addition and litter removal (F = 0.595; df = 1, 112; P = 0.595) (Fig. 4.4B).

Discussion

The results of our laboratory palatability trials suggest that many species of arthropod predator do not recognize *I. scapularis* as a prey item, or are unable to pierce their heavily sclerotized exoskeleton. Those predatory species which do feed on *I. scapularis* are significantly more likely to target engorged larvae than unengorged nymphs. Our findings also reaffirm the

observation originally noted by Carroll (1995) that *S. ocreata* readily targets *I. scapularis* under laboratory conditions. Furthermore, the results from our field microcosms suggest that this arthropod predator can strongly reduce the survival of questing *I. scapularis* nymphs.

We observed that two species of spider most commonly targeted *I. scapularis* in our laboratory palatability trials. As expected, *S. ocreata* targeted *I. scapularis* regardless of life stage (nymph / engorged larva). The other predatory species which readily targeted both life stages was *P. audax*, which is a species in the family Salticidae, and is common in the northeastern United States (Young and Edwards 1990, Wagner and Wise 1996). Both species are relatively large (> 3 mm), and they are both wandering hunters (Givens 1978, Cady 1983, Uetz et al. 2002). Even larger web building spiders were not generally found to target ticks in the palatability trials, and when *I. scapularis* was placed directly onto a web it was still mobile and the spider tended not to attack. Additionally, spiders that rely on suspended webs to capture prey are unlikely to come into regular contact with *I. scapularis* as ticks are not highly mobile, limiting the probability that they will encounter these webs. Many web builders also tend to target soft bodied prey (Clarke & Grant 1968, Lensing & Wise 2004, Günther et al. 2014), so they may lack the robust chelicerae needed to attack hard-bodied ticks. Overall, smaller species and individuals tended not to target *I. scapularis*, which could indicate that both size and hunting style are likely to affect whether predators recognize *I. scapularis* as a prey item.

Arthropod predators showed a general preference for engorged larvae over nymphs. This was particularly true for orb weaving species (Araneidae), and smaller spiders (appendix 1). The preference of predators and tick-targeting pathogens for engorged over flat ticks has been observed previously (Carroll 1995, Samish & Alekseev 2001) and may be due to the expansion of the tick endocuticle that exposes a large portion of the tick's body to attack, whereas flat ticks

are largely protected by their scutum (Beadle 1974, Hackman 1982, Randolph 2009). Furthermore, engorged ticks contain a large volume of protein-rich liquid, facilitating digestion, whereas a large portion of the biomass of flat ticks consists of an indigestible chitinous exoskeleton (Cohen 1998). The smaller protected area of engorged ticks may also increase their vulnerability to attack from smaller predators that may be able to puncture through exposed areas lacking heavy sclerotization. Additionally, little is known about the chemical defenses of *I. scapularis*, but these defenses occur in some tick species as well as other mites (Yoder 1995, Sakata & Norton 2001, Heethoff et al. 2011), and may differ between life stages. Our data suggest that large wandering spiders are most likely to target *I. scapularis*, and that tick life stage is a strong predictor of whether predators will generally view *I. scapularis* as a prey item. These findings have important implications for the development of integrated pest management protocols. Targeted timing of protocol deployment focused on the most vulnerable *I. scapularis* life stages could dramatically improve the effectiveness of IPM efforts.

The addition of *S. ocreata* to the microcosms significantly reduced the survival of *I. scapularis* over the 21-day trial period, with one-third lower survival relative to the reference microcosms (Fig. 4.2). Our litter removal treatment reduced the habitat complexity within the microcosms, which appears to have resulted in a weak interaction with the addition of predators. Perhaps leaf litter provided concealed refugia for *I. scapularis* allowing them to avoid attack by this wandering spider predator. It is also possible that the increased number of alternative prey items in the reference treatment slightly decreased the attack rate of *S. ocreata* on the ticks (Fig. 4.4). The reduction in tick survival reported here is not as large as those reported in field applications of *Metarhizium anisopliae* (Metchnikoff) (Benjamin et al. 2002), which is commonly used as a biopesticide (Stafford & Allen 2010), but our results indicate that *S. ocreata*

will target ticks in the presence of a complex litter and soil environment even when alternative prey items are present, making them an excellent candidate for use in integrated pest management protocols. Further research is required to determine the incidence of predation for *S. ocreata* under completely natural field conditions without the effect of an enclosed microcosm, and evaluate their potential to work in conjunction with biopesticides.

Our litter removal treatment also reduced the survival of *I. scapularis* independent of predator addition, but this reduction was much less dramatic than the effect of litter removal on tick density in previous studies (Schulze & Jordan 1995, Stafford et al. 1998). The exact mechanisms explaining large tick density reductions in litter removal studies has not been evaluated and could include the physical removal of ticks, or changes in microhabitat availability resulting in increased tick mortality. Our results imply that litter removal may cause only temporary dramatic reduction of tick populations that are not sustained as ticks recolonize the area. The effect of litter removal is also likely to be strongly affected by weather conditions. It rained during the period of the field study, and it is likely that the effect of litter removal would be stronger during drier periods (Stafford 1994, Berger et al. 2014). We also observed a secondary effect of soil organic matter content on tick survival; when leaf litter was removed % SOM was positively correlated with tick survival, but with litter present this relationship was not observed. Soil organic matter is known to increase microhabitat stability for many soil dwelling arthropods by decreasing variation in relative humidity and temperature (Vreeken-Buijs et al. 1998, Abu-Hamdeh et al. 2000) and appears to provide *I. scapularis* with additional protection when leaf litter is absent (Fig. 4.3). Localized litter removal may reduce contact rates between ticks and humans in high traffic residential areas (e.g. lawns and footpaths), but given the limited effect of litter removal we observed, the negative impact on non-target arthropods (Fig. 4.4), and

the difficulty involved in removing leaf litter from large areas, we don't believe that litter removal is an effective long-term solution for controlling populations of *I. scapularis* in forested ecosystems.

The predation of *I. scapularis* by soil-dwelling arthropods appears to depend upon the size and hunting style of the predator as well as *I. scapularis* life stage. We have also shown that a predator that targets ticks in the laboratory can impact the survival of *I. scapularis* under field conditions. Additionally, our findings suggest the importance of considering specific soil properties when evaluating how the density and survival of *I. scapularis* is affected by various landscape features. Tick-targeting spider species, including *S. ocreata* and *P. audax*, show potential as biological control agents which could be included in integrated pest management protocols for *I. scapularis* control.

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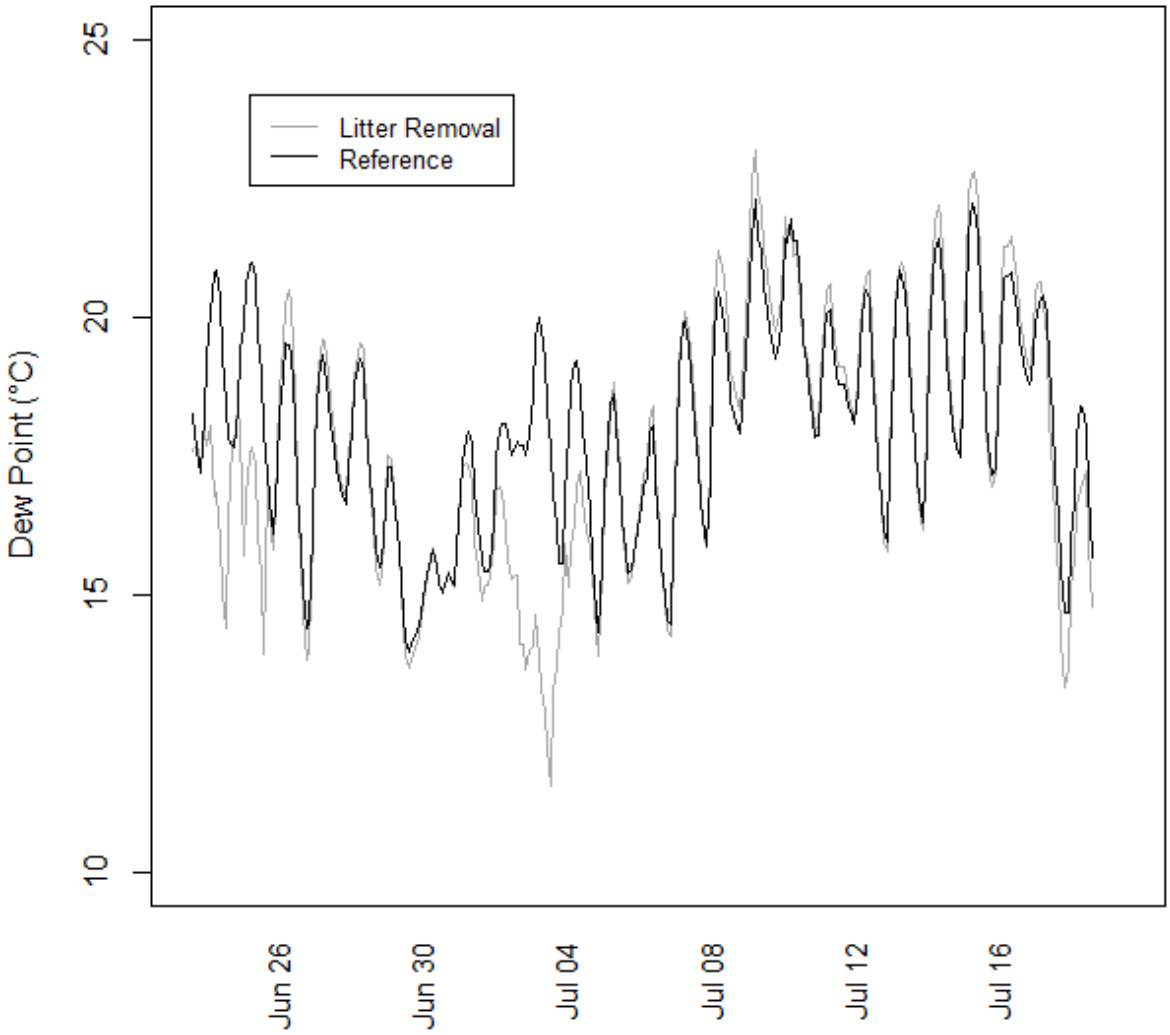

Figure 4.1: Dew points (°C) recorded by dataloggers in the microcosms at two hour intervals during the trial period between June 22nd 2015 and July 17th 2015. The dew points in the reference microcosms are in black, while those in the litter removal microcosms are grey. The lines represent the average of all dataloggers placed in their respective treatment across all three sites (n = 12).

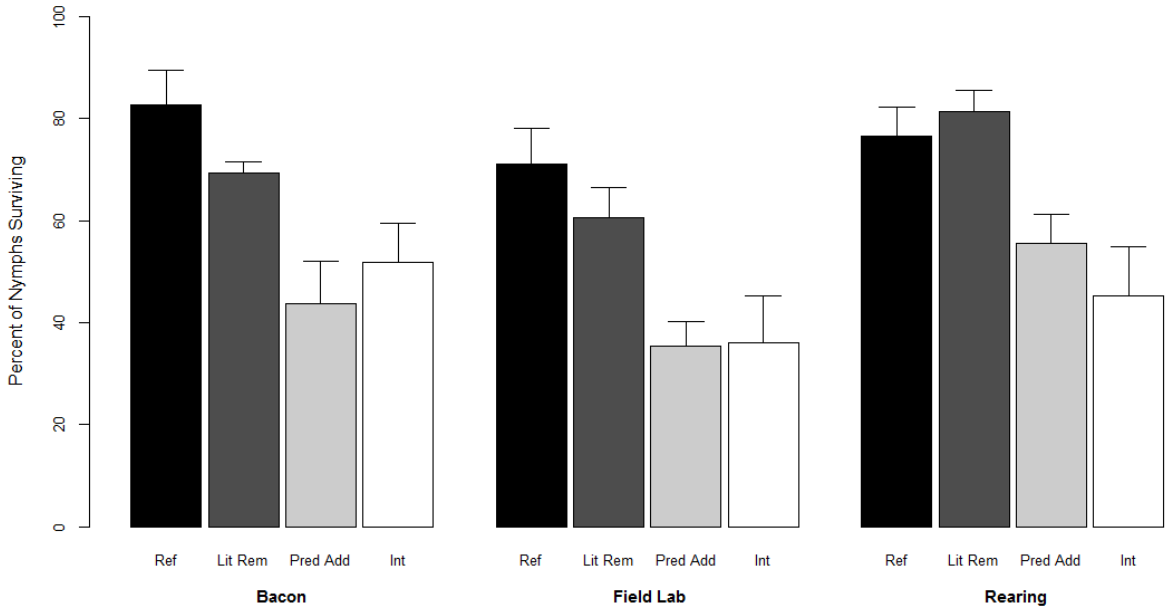

Figure 4.2: The mean (± SE) percentage of *I. scapularis* nymphs which survived after 21 days in the field microcosms. Data are divided into three field sites (Bacon Flats / Field Lab / Rearing Facility) and four treatment groups. The abbreviations for the treatments are as follows: 'Ref' = reference, 'Lit Rem' = litter removal, 'Pred Add' = predator addition, and Int = interaction between litter removal and predator addition.

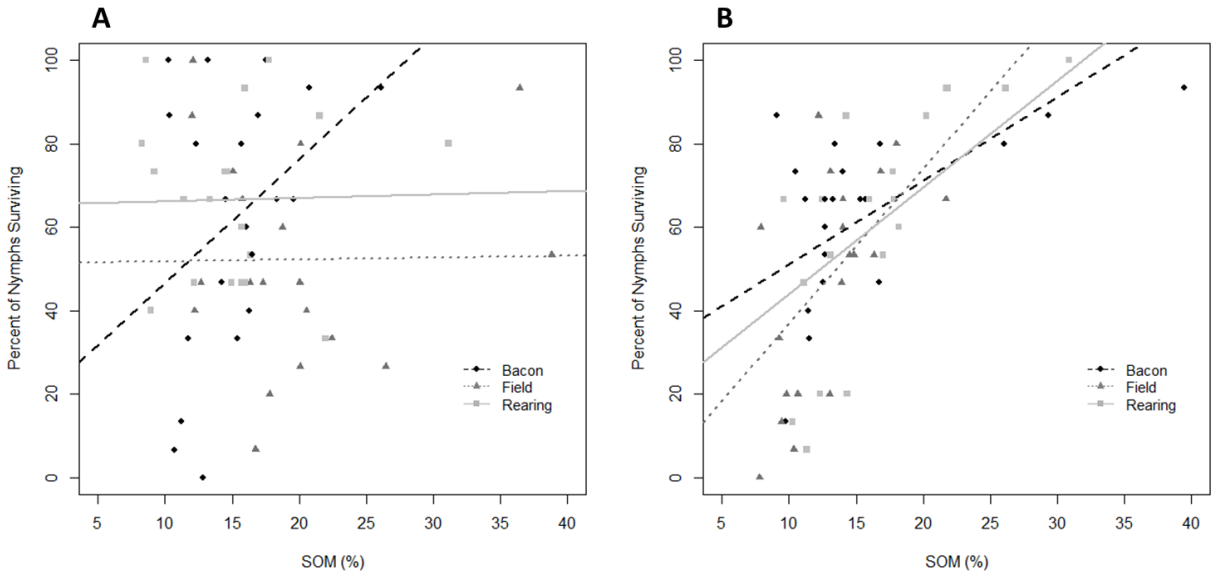

Figure 4.3: The relationship between the percentage of *I. scapularis* nymphs which survived after 21 days in the field microcosms and the percent soil organic matter (%SOM) of the soil in the microcosms. Figure A shows this relationship in the reference microcosms where leaf litter is present, and figure B shows the relationship in the microcosms with the leaf litter removed. Lines represent the line of best fit for each of the three field sites (Bacon Flats / Field Lab / Rearing Facility).

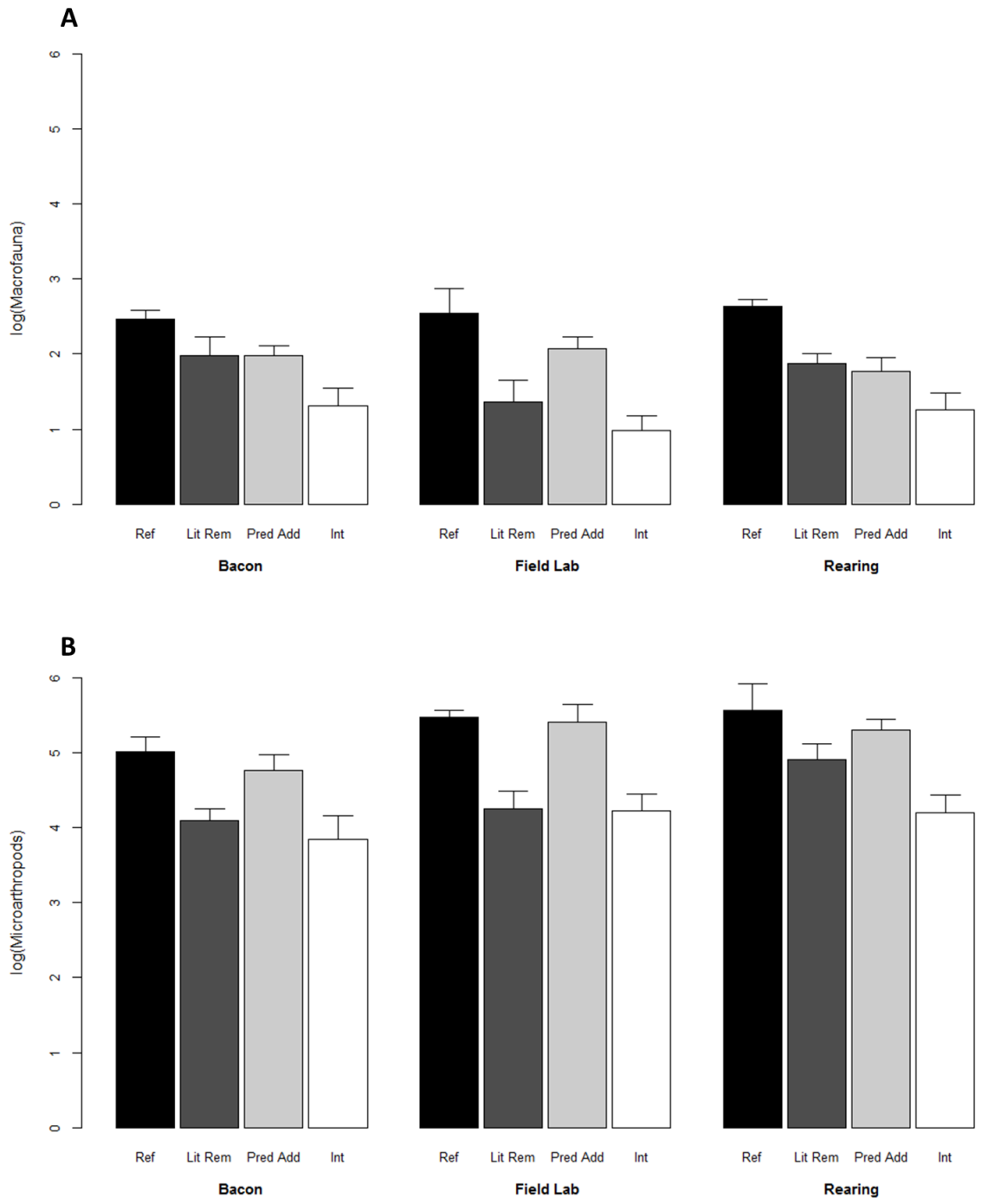

Figure 4.4: The mean (± SE) number of macrofauna and microarthropods present in the microcosms placed on the three field sites split between the four treatments. All arthropod data have been log-transformed.

Table 4.1: The percentage of nymphs or engorged larvae eaten by predators during the laboratory palatability trials. The predators have been divided into the 13 taxonomic families that were tested during the trials. The numbers in parentheses are the total number of individual trials that were performed in each taxonomic family. Detailed information regarding the predatory species tested is available in appendix 1.

Taxonomic Family	Nymphs Eaten	Engorged Larvae Eaten
Agelenidae	42.86 % (14)	50.00 % (2)
Amaurobiidae	0 % (8)	0 % (5)
Araneidae	11.11 % (18)	42.86 % (14)
Clubionidae	0 % (10)	33.33 % (6)
Eutichuridae	50.00 % (4)	100.00 % (2)
Geophilidae	0 % (18)	0 % (9)
Linyphiidae	0 % (11)	0 % (4)
Lithobiidae	14.71 % (34)	50.00 % (8)
Lycosidae	93.75 % (48)	94.74 % (19)
Miturgidae	0 % (6)	66.67 % (3)
Salticidae	26.19 % (42)	58.33% (12)
Theridiidae	0 % (7)	0 % (11)
Thomisidae	0 % (12)	11.11 % (9)
Total	**30.60 % (232)**	**40.35 % (104)**

Table 4.2: A list of the species and genera of arthropod predators tested during the laboratory palatability trials organized by taxonomic family. Under life stage 'EL' stands for engorged larvae and 'N' for nymph. The values in the % fed column represent the number of successful feeding events after 48 hours divided by the total number of trials for that species (n) expressed as a percentage.

Family	Genus	Species	Life stage	% Fed	n
Agelenidae	Agelenopsis	pennsylvanica	EL	50	2
			N	42.9	14
Amaurobiidae	Amaurobius	borealis	EL	0	5
			N	0	8
Araneidae	Araneus	marmoreus	EL	0	1
			N	0	2
	Araneus	pratensis	EL	NA	NA
			N	0	1
	Araneus	thaddeus	EL	33.3	3
			N	50	2
	Araniella	displicata	EL	NA	NA
			N	0	1
	Eustala	anastera	EL	75	4
			N	20	5
	Hypsosinga	rubens	EL	NA	NA
			N	0	1
	Mangora	placida	EL	50	2
			N	0	1
	Metepeira	labyrinthea	EL	100	1
			N	0	5
	Ocrepeira	ectypa	EL	0	3
			N	NA	NA
Clubionidae	Clubiona	abboti	EL	33.3	6
			N	0	10
Eutichuridae	Cheiracanthium	inclusum	EL	100	2
			N	50	4
Geophilidae	Geophilus	sp	EL	0	9
			N	0	18
Linyphiidae	Bathyphantes	pallidus	EL	0	4
			N	0	11
Lithobiidae	Lithobius	sp	EL	50	8

			N	14.7	34
Lycosidae	Schizocosa	ocreata	EL	94.7	19
			N	93.8	48
Miturgidae	Strotarchus	piscatorius	EL	66.7	3
			N	0	6
Salticidae	Evarcha	hoyi	EL	NA	NA
			N	0	10
	Habronattus	coecatus	EL	NA	NA
			N	0	1
	Naphrys	pulex	EL	0	4
			N	0	6
	Phidippus	audax	EL	100	7
			N	57.8	19
	Salticus	scenicus	EL	0	1
			N	0	6
Theridiidae	Asagena	americana	EL	0	5
			N	0	5
	Theridion	differens	EL	0	6
			N	0	2
Thomisidae	Xysticus	ferox	EL	11.1	9
			N	0	12

Table 4.3: Statistical readout of the mixed effects model exploring the effect of leaf litter removal, predator addition, and soil organic matter on the survival of *I. scapularis* nymphs in the field microcosms after the 21-day trial period. In addition to the fixed effects listed, field site was included as a random effect.

Parameters	*Df*	*F*	*P*-value
Predator Addition	1	47.53	< 0.001
Litter Removal	1	7.58	0.007
Soil Organic Matter	1	12.66	< 0.001
Predator x Litter	1	3.07	0.082
Litter x Soil Organic Matter	1	7.59	< 0.001
Site was included as a random effect in this model. Residual degrees of freedom = 110.			

Table 4.4: The AIC scores comparing the models of tick survival in the field microcosms. The null model included only 'site' as a random effect, the full model included all parameters and their interactions. The final model was the most parsimonious, and is detailed further in table 2. The ΔAIC scores represent changes from the AIC score of the null model.

Model	AIC	ΔAIC
Null Model	661.6	0
Full Model	603.8	-57.8
Final Model	604.9	-56.7

CHAPTER 5

SURVIVAL AND ENERGY USE OF *IXODES SCAPULARIS* DURING THEIR OVERWINTER PERIOD

Abstract

The blacklegged tick (*Ixodes scapularis*) spends approximately ten months in the soil between feeding as larvae and questing for hosts as nymphs the following year, and high tick mortality during this period has been observed. We tracked survival, energy use, and development of engorged larvae placed in field microcosms in northern hardwood forests from September to July during two field seasons (2013 / 2014 and 2015 / 2016) at two locations in New York State. In 2013, 85 microcosms were deployed at a site near Ithaca, NY with 15 engorged larvae placed in each microcosm. In 2015, 288 microcosms each with 15 engorged larvae were deployed across six field sites, half of which had high natural tick densities (> 12 nymphs / 150 m^2), whereas the other half were low-density (< 1.2 nymphs / 150 m^2) sites. In both years subsets of microcosms were destructively sampled periodically during the overwinter period to determine tick survivorship and physiological age. Neither energy use nor mortality differed significantly between sites with high vs low natural tick density, suggesting that cryptic, small-scale site related factors do not affect the survival of overwintering nymphs. However, across all sites, and in both years, tick mortality was higher in the spring than during the winter. Late spring weather conditions were much drier in 2016 than in 2014, and ticks collected in July 2016 had significantly lower lipid content and survival rates than those in July 2014. We also found a significant effect of soil organic matter (SOM) on the survival of *I. scapularis* during a

dry period in the spring of 2016, but this trend was not present during earlier collection periods, or in 2014. The finding that tick survival was significantly affected by SOM during dry periods suggests that organic matter may provide an environmental buffer for nymphs, especially when conditions are hot and dry. Our results suggest that *I. scapularis* nymphs experience their highest levels of mortality in the spring and early summer, due partially to depletion of energy reserves. Additionally, our data suggest that hot, dry weather conditions in the spring and early summer reduce nymphal survival and energy storage.

Introduction

Generally, the lifecycle of most non-nidicolous, multi-host ticks, including *Ixodes scapularis* the primary vector for several tick-borne diseases including Lyme disease (Nelson et al. 2015), can be considered a war of attrition. Female *I. scapularis* lay egg masses containing up to 3,000 eggs (Mount et al. 1997), of which < 1% generally survive to reproduce (Awerbuch & Sandberg 1995, Wu et al. 2013). Survival rates vary for a multitude of reasons (Ogden et al. 2014), including host contact rates and environmental stressors. Many tick species from northern latitudes spend a large portion of their life cycle in diapause to avoid cold temperatures during the winter (Sonenshine & Roe 2013). Ticks in winter diapause exhibit low metabolic rates (Belozerov 1982), but mortality during this period has rarely been monitored. *I. scapularis* spends over 95% of its two-year life cycle in the soil either questing for hosts or in diapause (Ostfeld & Brunner 2015). The longest inactive period in the *I. scapularis* life cycle occurs between the larval and nymphal life stages and lasts approximately from September to June (Ogden et al. 2004). Mortality during this period (Ginsberg & Zhioua 1996) is thought to depend largely upon the probability of ticks encountering hosts on which to feed during their larval questing period in late summer (Ogden et al. 2005). However, feeding alone does not account for the attrition that occurs between the larval and nymphal life stages (Lindsay et al. 1998), and the non-host related factors responsible for this excess mortality have received limited attention (Eisen et al. 2016).

A variety of off-host factors are known to affect the densities of questing *I. scapularis*, including weather conditions, particularly in winter and spring (Schulze & Jordan 2003, Ogden et al. 2006, Berger et al. 2014, Burtis et al. 2016a). Research regarding the off-host dynamics of *I. scapularis* has focused primarily on their actively questing life stages (Jones & Kitron 1999,

Ostfeld et al. 2006), or absolute overwinter survival (Lindsay et al. 1995, Ginsberg & Zhioua 1996, Burtis et al. 2016b). Because of the long duration of the overwintering period for *I. scapularis* nymphs (Lindsay et al. 1998), the timing of mortality events is uncertain (Brunner et al. 2012). Furthermore, mortality may occur rapidly from exposure to harsh conditions like desiccation and temperature extremes (Stafford 1994) or these conditions may cause gradual increases in mortality due to depletion of tick energy reserves (Hermann & Gern 2012). Although some researchers have measured the body condition of ticks collected from the field (Randolph & Storey 1999), the linkage to mortality throughout the ten-month nymphal overwintering period under field conditions has not been explored.

Measurements of physiological age have proven to be most reliable for representing the body condition of ticks (Upensky 1995). In the most common methods lipids are extracted from the tick cuticle to measure energy storage, which is strongly correlated with age and survival for both *I. scapularis* (Pool et al. 2017) and its close relative *I. ricinus* (Steele & Randolph 1985, Herrmann et al. 2013). Pairing this physiological age measurement with an overwinter *I. scapularis* mortality curve under field conditions would allow for the determination of when *I. scapularis* nymphs experience their highest levels of mortality, and whether mortality is associated with physiological stressors (drought / cold). Additionally, the timing of pulses of tick mortality likely varies interannually in response to weather conditions, as well as possible variation in biotic factors within the soil environment, including predator density and the prevalence of entomopathogens (Samish et al. 2004, Burtis & Pflueger 2017).

Densities of questing *I. scapularis* nymphs show a high degree of spatial heterogeneity at fine spatial scales (Pardanani & Mather 2004), presumably reflecting spatial heterogeneity in soil properties, such as litter depth (Lubelczyk et al. 2004, Clow et al. 2017), soil texture (Guerra et

al. 2002), soil organic matter content, and microclimate (Lindsay et al. 1999) that determine the quality of tick habitat (Boehnke et al. 2017). The distribution of high-quality tick habitat, both between and within forest plots, is likely to have a strong effect on overwinter nymphal survival rates due to their low mobility (Bertrand & Wilson 1996, Ostfeld et al. 1996), but this effect has not been directly observed under field conditions.

Biotic interactions also may affect the survival of *I. scapularis*, including entomopathogens (Kirkland et al. 2004) and arthropod predators (Burtis & Pflueger 2017). Predators and pathogens can have a strong impact on the distribution of many other soil-dwelling biota (Roy et al. 2006, Lenoir et al. 2007, Quesada-Moraga et al. 2007), but they have received limited attention in tick density studies. The distribution of tick-targeting entomopathogens is not well-characterized (Tuininga et al. 2009) and may play a role in the patchy spatial distribution of *I. scapularis*. Additionally, at local scales many areas with suitable tick habitat remain uncolonized, likely due to host community dynamics (Hahn et al. 2016), but the capacity of ticks to colonize these areas remains uncertain.

Thus, the objective of the present study was to evaluate factors regulating the overwinter survivorship of nymphal *I. scapularis* in northern hardwood forests. Here we focus on ticks in the lower Hudson Valley in New York State, a region with particularly high incidences of those tick-borne diseases vectored by *I. scapularis*, most notably Lyme disease (Diuk-Wasser et al. 2012). By measuring survivorship in microcosms across several forest sites exhibiting low and high natural tick density, and in two years with contrasting weather we aimed to test the hypotheses that tick survivorship is 1) lower on low-density than high-density sites, and 2) varies between years as a result of particular weather-related stressors, especially hot, dry conditions. Moreover, by examining the body condition of ticks recovered from the microcosms we hoped to

test the hypothesis that the majority of nymphal mortality would occur late in their overwintering period, due to a decline in energy storage. Because our sites were well-matched in terms of slope, soil type, forest composition, and understory vegetation, we expected no differential effect of microhabitat availability on tick survival, but within each site we expected ticks inhabiting low-quality habitat to show higher mortality over time due to reduced protection from weather conditions.

Methods

Field Site Descriptions

Research in 2015 – 2016 was conducted on six field sites located in Dutchess County in New York State. We used records from 194 sites, upon which tick density dragging data were collected in 2011 and 2012 by Dr. R. Ostfeld, to locate field sites with high vs low natural tick density (Table 5.1). During these collections all sites were sampled using the dragging method (Schulze et al. 1997). A 120 m x 120 m sampling grid, or an irregular grid covering the same total area (14,400 m^2), was established on each site. Sites were visited twice per year. During each visit 16 transects were sampled, and drag cloths were checked every 30 m (480 m^2 per visit). Tick densities were low across the majority of the collection sites in 2012, so we used the 2011 data to identify high- and low-density sites. During this dragging period high-density sites averaged >12 nymphs per 100 m^2 during the nymphal seasonal peak, whereas low-density sites averaged < 1.2 nymphs per 100 m^2. The high-density sites will be referred to hereafter as Cary (41°48'8.71"N ; 73°44'29.71"W), Tymor (41°38'40.76"N ; 073°41'37.80"W), and Wilcox (41°56'54.03"N ; 73°43'22.51"W). The low-density sites were named Depot (41°34'19.73"N ; 73°40'49.18"W), Sharpe (41°30'35.66"N ; 73°52'1.02"W), and Taconic (41°58'11.42"N ; 73°30'4.83"W). The minimum distance between these sites was 7.0 km and some sites used for

our experiment were moved to nearby locations within the same forest fragment, ensuring that all sites were well-matched for slope, soil type, forest composition, and understory vegetation.

All sites were characterized as second-growth northern hardwood forests where the canopy was dominated by sugar maple (*Acer saccharum*) and red oak (*Quercus rubra*). All stands were between 70 – 100 years old, with the exception of the Taconic site that was located in a younger stand at 50 years old. The soils at five of the sites were classified as either Nassau-Cardigan or Hollis-Chatfield series and were rocky Inceptisols, with nearly level (0 – 5%) slopes. Soil at the Taconic site was a Copake gravelly silt loam (USDA 2017). All sites were selected to have little understory vegetation cover, limiting the effect of variation in the potential protective effects of understory cover between sites (Williams & Ward 2010). A 30 x 30 m sampling and randomization grid with flags every 3 m was established on each of the sites. A 30 x 30 m sampling and randomization grid with flags every 3 m was established on each of the sites. These 121-point grids were used to randomly select the locations of microcosms and determine which transects were to be dragged to collect tick density data in 2015 and 2016.

For interannual comparisons, overwintering tick survival data in 2013 – 2014 were collected at one site located in Ithaca NY (42°28'4.06"N ; 76°25'34.21"W). This site was also located in a northern hardwood forest dominated by *A. saccharum* on a Howard gravely loam soil with no understory vegetation. A 136-point sampling grid was established on this site, with points separated by 3 m (21 m x 48 m). This grid was used to randomize the locations of the microcosms.

Tick Density Data

Larval and nymphal *I. scapularis* density data were collected weekly across the six sites in Dutchess County during the summer (May – August) in both 2015 and 2016 using the tick

dragging method (Schulze et al. 1997). This allowed for high-resolution temporal data of natural tick activity on the sites during the study period, and ensured that the density trends observed in 2011 were not temporary. Ticks were collected weekly between May 20th and September 1st by dragging a 150 m^2 area on each site. Drag cloths were checked every 30 m, and five 30 m transects were randomly selected using the grids laid out on each site. Ticks were collected into vials containing 70% ethanol and returned to the laboratory where they were keyed to species (Durden & Keirans 1996). Sites were dragged between 8:00 AM – 6:00 PM, and drag times were alternated to ensure that no site was repeatedly sampled during the same time of day.

Larval I. scapularis Collection and Rearing

We collected the larval *I. scapularis*, for addition to the microcosms, in July and August of 2013 and 2015 using the dragging method. These larvae were collected for both years (i.e. 2013 / 2015) on Cary Institute of Ecosystem Studies (CIES) property (41°46'56.91"N ; 73°43'59.18"W) in an area with a dense understory of Japanese Barberry (*Berberis thunbergii*). After collection, the larvae were fed upon lab-raised mice (*Peromyscus leucopus*) within 6 hours. A total of 100 larvae were placed on each mouse using a fine tipped brush. Once inoculated, mice were placed in a small PVC tube with food for 4 hours to discourage grooming and allow the ticks to begin feeding. Mice were then kept in a wire mesh cage suspended above a tape-lined tray with a moistened towel in the bottom as described in Keesing et al. (2009). The trays were checked every twelve hours and engorged larvae were collected and deposited into humidified vials, which were kept at 20 °C for no more than three weeks before being placed in the field. Prior to deployment, larvae were randomly assigned to microcosms and field sites to ensure that microcosms would not contain ticks that were collected and fed during the same time period or on the same host. All mice used in this experiment were obtained from the Peromyscus genetic

stock center supported by the University of South Carolina, and maintained in a facility at the CIES. All animal handling procedures were approved by a joint IACUC protocol (2013-0015) between the CIES and Cornell University.

Microcosm Deployment

Overwinter survival was studied in the field using microcosms containing soil and leaf litter from the field sites to ensure that ticks had access to natural refugia. To construct each microcosm a PVC segment (15 cm diameter / 5 cm depth) was placed on the surface of the soil, and a knife was used to cut the soil and leaf litter inside the core. This allowed the PVC segment to be pushed into the soil. The segment containing a soil core was then lifted using a spatula and placed inside a fine mesh organdy bag. At this point 15 engorged larval *I. scapularis* were added to the bag using a fine brush. The bag was then sealed using a cable tie and the microcosm was placed back into the original hole in the soil. The microcosm was then lightly covered with leaf litter from the surrounding area to avoid animal disturbances during its field deployment. In 2015 microcosms were deployed between August 25th and 29th across all six sites, while in 2013 all microcosms were deployed on September 1st.

In 2015 an additional four microcosms containing HOBO U23 pro V2 dataloggers were installed at each site to record temperature and relative humidity within the microcosms throughout the study period, allowing us to directly relate tick survival and physiological condition to the environmental conditions within the microcosms. Data loggers were placed at the interface under the leaf litter on the surface of the mineral soil in each microcosm. These data were used to calculate vapor pressure deficit (VPD) in 2015 – 2016 (Murray 1967, Monteith & Unsworth 2007). Air temperature and precipitation data were collected from two NOAA weather stations, one located in Millbrook, NY (ID # USW00064756) and one in Ithaca, NY (ID #

USC00304174) (NOAA 2017), and used to compare weather conditions between the two field seasons (2013 – 2014 / 2015 – 2016). We used these data to calculate the number of hot (T > 25 C) dry (Precip = 0) days (HDD) throughout the two field seasons, a metric that has been found to correlate negatively with both tick activity and Lyme disease incidence (Burtis et al. 2016a).

Tick Recovery and Lipid Extraction

In 2015 – 2016 a subset of the microcosms was collected from the field on the following six days; 1) October 18th 2015, 2) December 6th 2015, 3) January 29th 2016, 4) March 19th 2016, 5) May 12th 2016, and 6) July 2nd 2016. A total of 48 randomly-selected microcosms were collected on each day (eight per site). During the 2013 – 2014 field season there were three collection days, December 16th 2013, February 15th 2014, and July 5th 2014. A total of 20 microcosms were retrieved during the December and February collections, and 45 were collected in July. Microcosms sat at room temperature for 48 hours, and then all ticks were collected over three days. Collection involved hand sorting the leaf litter and soil for 30 minutes, and then hanging the materials in a Berlese funnel for three days under a 25-watt light bulb. This method is effective for collecting both nymphs and engorged larvae from microcosms (Burtis 2017). After the ticks were extracted, the soil was removed from the funnel, homogenized, and dried at 65 °C for 48 hours. The soil was then weighed and placed in a muffle furnace at 400 °C for four hours and then reweighed to determine loss on ignition, which was used to estimate the soil organic matter concentration (%SOM).

The lipid index of the nymphs was determined using a chloroform extraction method which has been shown to be effective for *I. scapularis* (Pool et al. 2017). Ticks were collected from the ethanol jars at the bottom of the Berlese funnels every 24 hours to reduce interference with the lipid extraction values, as ethanol can dissolve lipids. We tested the effect of storage in

ethanol on lipid index values by placing a set of ticks in ethanol for 0, 1, and 7 days and then using the chloroform lipid extraction method. Ticks that spent 24 hours in ethanol had a slight (< 8%), but non-significant reduction in lipid content (Fig. 5.1). Therefore, to standardize ethanol contact all ticks collected from the microcosms spent 24 hours in ethanol. In addition to the ticks collected from the field, 100 engorged larvae were kept under laboratory conditions during both years in order to determine their molting success, and the initial lipid index values of the molted nymphs.

To estimate lipid content and physiological age, nymphs were dried at 70 °C for 48 hours, weighed, and then placed in chloroform for 72 hours, with the chloroform being exchanged every 24 hours. Nymphs were weighed as a group from each microcosm to ± 1 µg using a Sartorius MC5 Microbalance; thus, we obtained an average physiological age of the nymphs within each microcosm. We calculated the 'lipid index' to correct for the increased lipid storage of large bodied individuals. In order to calculate the index, we determined the average body and lipid mass of all the ticks within each microcosm. We then took the square root of the average lipid mass and divided it by the average body mass of ticks within each microcosm (Steele & Randolph 1985).

Statistical Methods

We constructed a mixed effects model to analyze the natural density of questing ticks on our six sites in Dutchess County in order to validate our density treatment and compare natural tick densities between years. This model included site tick density (high / low), year (2015 / 2016), and collection date as fixed effects, with site as a random effect. A linear mixed effects model was used to detect the relationship between tick survival in the microcosms and lipid index values; because collection date and lipid index were strongly correlated we included

collection date as a random effect nested within site, which was nested within year. Any microcosm that did not contain nymphs at the time of collection was not included in our analyses of lipid index. We ran a mixed effects model to determine the effect of collection date and % SOM on tick survival, and a second mixed effects model was constructed to evaluate the effect of collection date on the lipid index values. Both models included 'site' as a random effect. The potential fixed effects for these models were natural tick density at the site, collection date (as a categorical variable), and % SOM. Inclusion of terms for both models was determined according to their AIC scores (Tables 5.2 & 5.3). Post-hoc Tukey tests were also run on both models allowing for direct comparisons between collection dates to determine when ticks experienced significant changes in survival and lipid index values. The p-values were corrected for multiple comparisons using the Holm–Bonferroni method. Vapor pressure deficit (VPD) measurements were averaged across all six sites and then categorized by collection period. We used an ANOVA to detect overall differences in VPD between the six collection periods in 2015 – 2016.

To evaluate whether the survival and physiological age of *I. scapularis* nymphs differed significantly between the two field seasons (2013 – 2014 / 2015 – 2016) we ran two mixed effects models. Only collections two, three, and six from the 2015 – 2016 field season were included in these analyses, as they closely matched the collection dates from the 2013 – 2014 field season. Potential fixed effects for these models included collection date, year of collection, and SOM as well as interactions between these factors. AIC scores were used to select parameters (Tables 5.4 & 5.5) and site was included in both models as a random effect**.** We recognize that effects cannot be conclusively ascribed to interannual variation because the studies were conducted on different field sites, and we evaluate this further in the discussion. We also ran a t-test to compare the physiological age of the nymphs that molted under laboratory

conditions in 2013 versus 2015. Additionally, a generalized additive model was used to compare the number of HDD per week between the two years. This model included year as a factor, and week as a smoothing term to detrend the temporal increase in HDD within each year. For all models, procedures described in Zuur et al. (2009) were followed when selecting factors using AIC values. All analyses were conducted using the R statistical software.

Results

The density of *I. scapularis* was significantly higher on the high-density sites than the low-density sites for both questing larvae ($F = 36.32$; $df = 1, 46$; $P < 0.001$) and nymphs ($F = 69.66$; $df = 1, 46$; $P < 0.001$). Densities of larvae ($F = 8.23$; $df = 1, 46$; $P = 0.006$) and nymphs ($F = 11.89$; $df = 1, 46$; $P = 0.001$) were significantly higher in 2016 than 2015 (Fig. 5.2). Densities (expressed per 150 m^2) of *I. scapularis* in 2015 on the low-density sites peaked at 1.7 nymphs and 4.7 larvae, and in 2016 they peaked at 0.7 nymphs and 0.7 larvae. On the high-density sites the peak nymphal density in 2015 was 14.0 and larval density peaked at 39.7, while in 2016 the density of nymphs was 5.7 and larvae was 13.0. Accordingly, 14.9% of larvae questing in 2015 survived to quest as nymphs the following year (2016) on the low-density sites, whereas 14.4% survived on the high-density sites.

Few engorged larvae were recovered from the microcosms as over 99% of the surviving ticks recovered had molted into nymphs in the field. Of the 100 engorged larvae allowed to molt under laboratory conditions during each year 95% successfully molted into nymphs in 2014, while 94% molted in 2016. The lab-reared nymphs also had similar lipid index values indicating no difference in initial body condition between years (2013 / 2015) ($t = -0.50$; $df = 38$; $P = 0.620$). In the field, there was a positive correlation between the number of nymphs surviving within a microcosm and their lipid index values ($t = 4.64$; $df = 145$; $P < 0.001$).

No significant difference was observed in tick survival during any of the collections between the high vs low-density sites (Fig. 5.3). Although we found a significant relationship between % SOM and tick survival, the relationship was only significant during the final two collection periods in 2016 on day 258 ($t = 3.90$; $df = 265$; $P < 0.001$), and day 309 ($t = 4.99$; $df = 265$; $P < 0.001$) (Table 5.6) (Fig. 5.4). This relationship did not appear during the 2013 – 2014 collections ($t = 0.779$; $df = 109$; $P = 0.473$), and % SOM was excluded from our analyses of variation in nymphal lipid content according to the AIC values (Table 5.5). In 2015 – 2016 VPD varied significantly among collection periods ($F = 55.83$; $df = 5, 303$; $P < 0.001$) increasing during the fourth collection period and peaking during the final two collection periods (Fig. 5.5). Tick survival ($F = 27.75$; $df = 5, 265$; $P < 0.001$) and energy storage ($F = 63.81$; $df = 5, 245$; $P < 0.001$) both varied significantly with collection date, decreasing significantly during the fourth collection, and continuing to decrease further through the final two collections (Fig. 5.3).

During the 2013 – 2014 field season at Ithaca, NY the number of hot ($T < 25$ °C) dry ($Precip = 0$) days (HDD) was significantly lower than in 2015-2016 in Dutchess County ($F = 120.6$; $df = 1, 3685$; $P < 0.001$) (Fig. 5.6). The number of nymphs surviving was significantly higher at the final collection in 2014 than for the same collection period in 2016 ($t = -3.78$; $df = 208$; $P < 0.001$), but survival did not differ significantly during our collections in December or February (Table 5.7) (Fig. 5.7). Similarly, the physiological age of the ticks did not differ significantly between years in December or February (Table 5.8), but nymphs collected in July of 2016 had significantly lower lipid index values than those collected during the same time period in 2014 ($t = -2.84$; $df = 194$; $P = 0.005$) (Fig. 5.7).

Discussion

Despite natural tick densities on the high- versus low-density sites in Dutchess County differing by nearly an order of magnitude, we observed no significant difference between the two density categories (high / low) in the survival or lipid index values of *I. scapularis* collected from our microcosms. Thus, small-scale, site related factors apparently did not affect the survival of *I. scapularis* during their nymphal overwintering period. At fine scales, spatial variation in tick densities tends to be heterogeneous, and without spatial autocorrelation (Pardanani & Mather 2004). Whether this heterogeneity results from host community dynamics or site factors directly impacting tick off-host survival is unknown. Additionally, correlations between tick density and landscape factors that have been described in observational data sets show high variability (Killilea et al. 2008), suggesting the effects of unmeasured factors. Our results do not indicate that non-host related cryptic factors differentially affected tick survival during the nymphal overwintering period, but further research is needed to investigate other life stages, particularly because many entomopathogens of ticks are life stage dependent (Gindin et al. 2002, Hornbostel et al. 2005).

We found that attrition in the natural tick population, with about 15% of questing larvae surviving to quest as nymphs the following year, closely matched survival in the microcosms at the time of our final collection (16.5%). Because the microcosms excluded hosts, the similarity of these values suggest that off-host factors strongly regulate the mortality of *I. scapularis* between their larval and nymphal life stages. The probability of finding a host is often a strong factor affecting tick survival during this period (Levin and Fish 1998, Ogden et al. 2005), but not always (Schmidt et al. 1999), and the host-mediated effect may have been overpowered by the drought conditions in late spring 2016. The effect of hot, dry conditions on tick survival is also reflected in comparisons between VPD and nymphal survivorship in the microcosms during

spring 2016. Ticks began to experience significantly higher mortality in March, when VPD also began to increase. This trend is also reflected in the lipid index values, with energy storage being dramatically reduced during the final two collections (Fig. 5.5). The *I. scapularis* nymphs also experienced high levels of mortality during their molt from engorged larvae, with 31% dying between their initial placement into the microcosms and the first field collection. Under laboratory conditions 94% of the engorged larvae molted successfully, but the relatively high mortality in the field suggests that *I. scapularis* is particularly vulnerable when molting under field conditions.

Interestingly, survival and lipid index values did not change significantly during the winter, with ticks collected from our microcosms in December and February exhibiting no significant difference in survival or lipid index values (Fig. 5.3). Low energy usage during the winter months is likely due to the nymphs entering diapause to reserve energy during this physiologically stressful period (Yuval & Spielman 1990). The lack of cold-related stress on *I. scapularis* is likely due to their high level of cold tolerance (Vandyk et al. 1996, Burks et al. 1996), combined with the high degree of protection afforded to ticks by the soil environment (Boehnke et al. 2017). Our results add to the growing body of evidence that cold winter temperature does not negatively impact *I. scapularis* survival in much of the northeastern United States and Canada (Lindsay et al. 1995, Brunner et al. 2012, Ostfeld & Brunner 2015, Ogden et al. 2016, Burtis et al. 2016b). Our detailed overwinter survival curve shows that *I. scapularis* experiences high levels of mortality in the spring and early summer, which could explain previously observed negative correlations between questing *I. scapularis* densities and spring weather conditions (Berger et al. 2014, Burtis et al. 2016a).

The comparisons of tick survival in our microcosms between the two field seasons (2013-2014 / 2015-2016) provide additional tentative evidence that drought conditions have a strong effect on *I. scapularis* survival and energy storage. At the end of 2013 – 2014 overwinter survival and lipid index values were significantly higher than those in 2015 – 2016 (Fig. 5.7). Comparisons between these two field seasons suggest that there is an increase in mortality during hot, dry conditions driven by rapid depletion of energy reserves. This energy loss may be a physiological factor driving the early seasonal emergence of *I. scapularis* nymphs during warm weather conditions (Levi et al. 2015, Monaghan et al. 2015). We emphasize, however, that our interannual comparisons are limited by the fact that these collections occurred on different sites; although we controlled for known site factors that might affect the overwinter survival of *I. scapularis*, we cannot fully account for site-dependent factors. Further research regarding the effect of drought on tick physiology and interannual survival rates is needed. Much of our knowledge regarding the effects of drought on tick populations is based upon natural densities of questing ticks (Berger et al. 2014, Burtis et al. 2016a), but these data can be strongly affected by tick behavior (Vail and Smith 1998), such as reduced questing activity to avoid desiccation during hot, dry conditions (Schulze et al. 1997, Perret et al. 2003). Microcosm studies remove this confounding factor and will improve our ability to understand the direct effect of drought on *I. scapularis* population dynamics and phenology.

We also observed a significant effect of soil organic matter on the overwinter survival of *I. scapularis* nymphs. Ticks placed in microcosms that contained highly organic soils exhibited lower overwinter mortality, but this effect only emerged during our final two collections in 2016 (Fig. 5.4) and was not present in any of our collections in the wetter year, 2014. Perhaps SOM provided a protective effect for ticks during dry conditions in the spring and early summer of

2016. Soil organic matter provides microhabitats with a more stable physical environment, protecting many soil-dwelling organisms from harsh climatic conditions (Vreeken-Buijs et al. 1998, Christenson et al. 2017). Additionally, soil organic matter has previously been observed to affect the survival of questing *I. scapularis* nymphs when leaf litter is absent (Burtis & Pflueger 2017). Soil organic matter content was similar across our Dutchess County sites; hence, we were unable to evaluate site-level effects on tick survival, but SOM can vary with forest and soil type, and may in part drive variation in tick densities at a localized scale. In summation, these patterns suggest that soil organic matter plays an important part in tick survival, particularly during drought conditions.

Conclusions

Our results indicate that *I. scapularis* nymphal overwinter mortality is highest in the spring and early summer, and is largely driven by the depletion of energy reserves. We also tentatively suggest that tick mortality and energy usage are higher during hot, dry spring conditions whereas cold winter temperatures have limited effect. The lack of relationship between natural *I. scapularis* densities and overwinter survival suggests that the fine-scale spatial patterns of *I. scapularis* nymphal populations are not controlled by cryptic off-host factors. The relationship observed between soil organic matter and tick survival in our microcosms suggests an important protective effect against drought with the potential to affect *I. scapularis* spatial distributions during drought periods. We focused on the ten-month period between the *I. scapularis* larval and nymphal activity peaks, but the effect of these factors must also be investigated for other *I. scapularis* life stages. Our data suggest that spring weather conditions strongly affect the survival and body condition of *I. scapularis* nymphs. Ultimately, spring weather has the potential to impact tick populations and questing behavior, thereby affecting

tick-host contact rates and the transmission dynamics of tick-borne pathogens in the northeastern United States.

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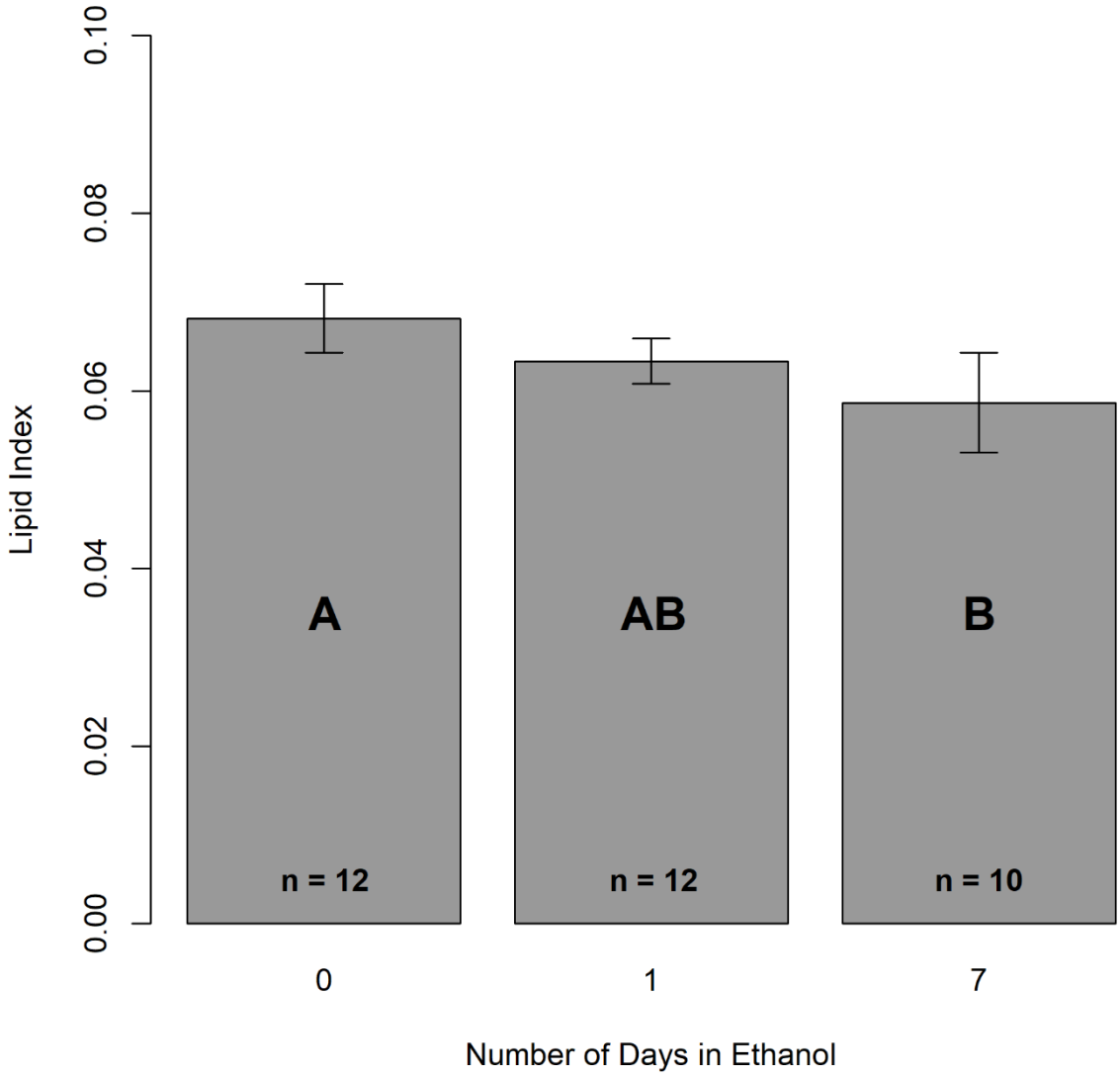

Figure 5.1: Mean and 95% CI of the lipid index for ticks kept in ethanol for 0, 1, and 7 days. Letters represent significant differences ($p < 0.05$) according to an ANOVA and post-hoc tukey HSD test for multiple comparisons. Each replicate above contained five *I. scapularis* nymphs.

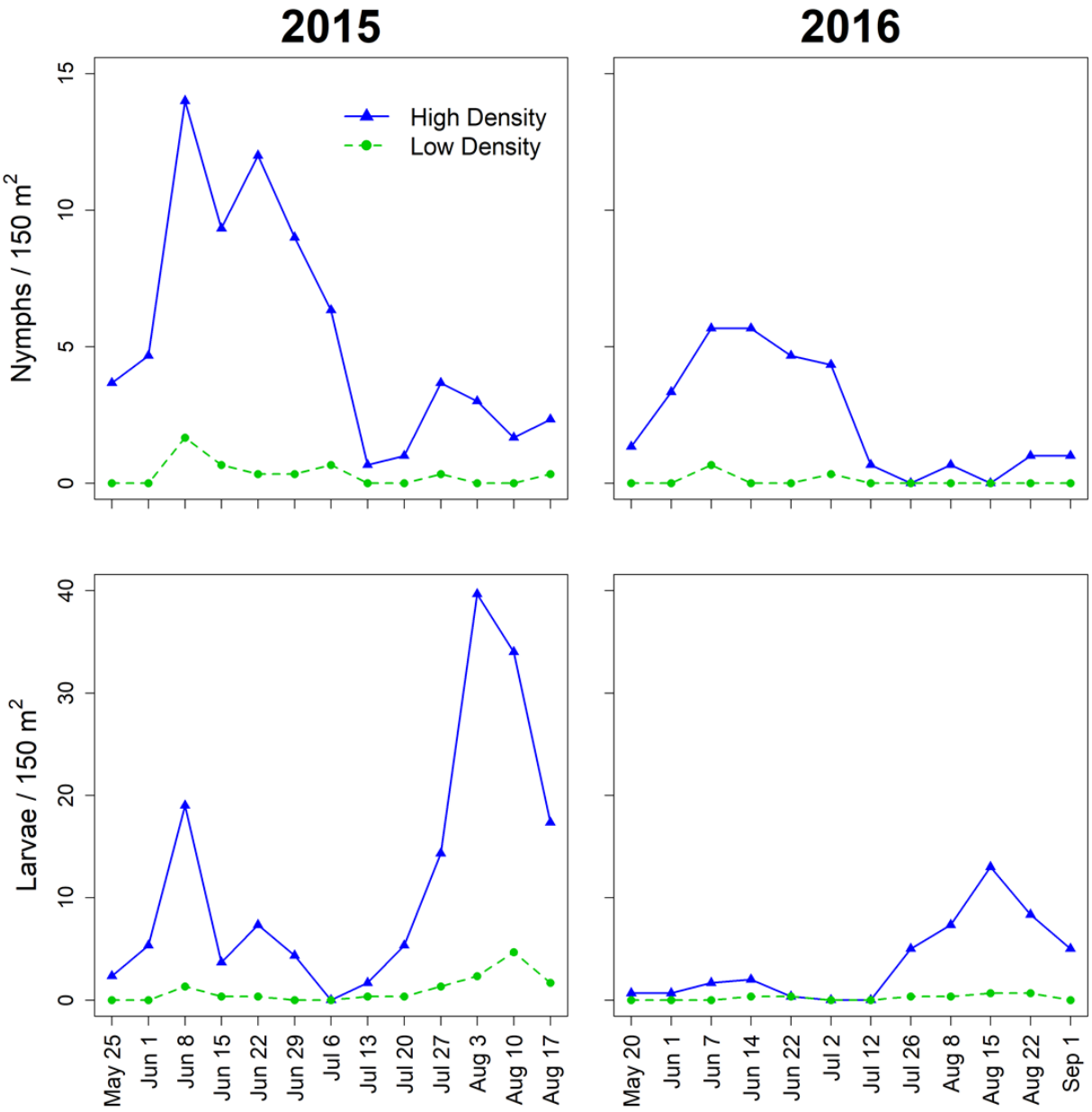

Figure 5.2: The density of questing nymphs (top) and larvae (bottom) in 2015 (left) and 2016 (right) for the high (solid lines) and low (dashed lines) density sites. The points represent the average density on the given collection date for the three sites in that category. All densities are presented as the number of ticks per 150 m^2.

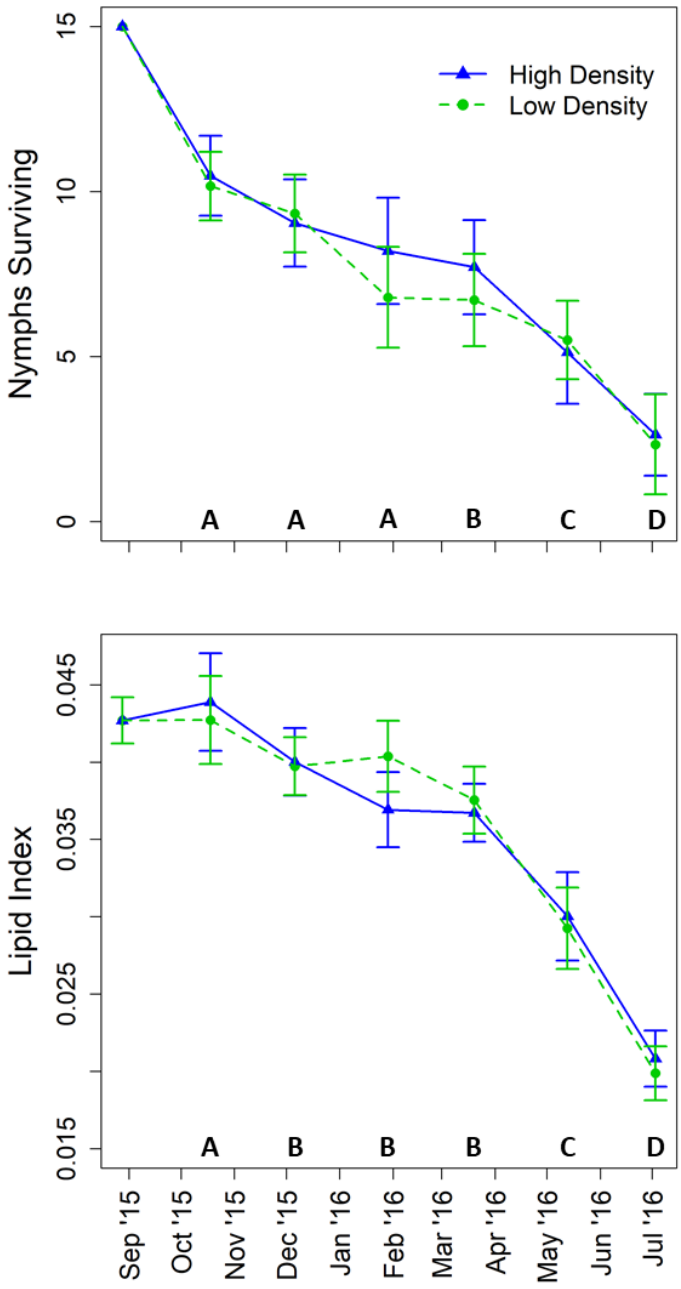

Figure 5.3: Survival and physiological age of the nymphal *I. scapularis* collected from the microcosms in 2015 – 2016. The points represent the means for the high (solid lines) and low (dashed lines) density sites, and the error bars show the 95% confidence interval for each collection date. Letters represent those time periods which differ significantly ($P < 0.05$) from one another according to the post-hoc Tukey test.

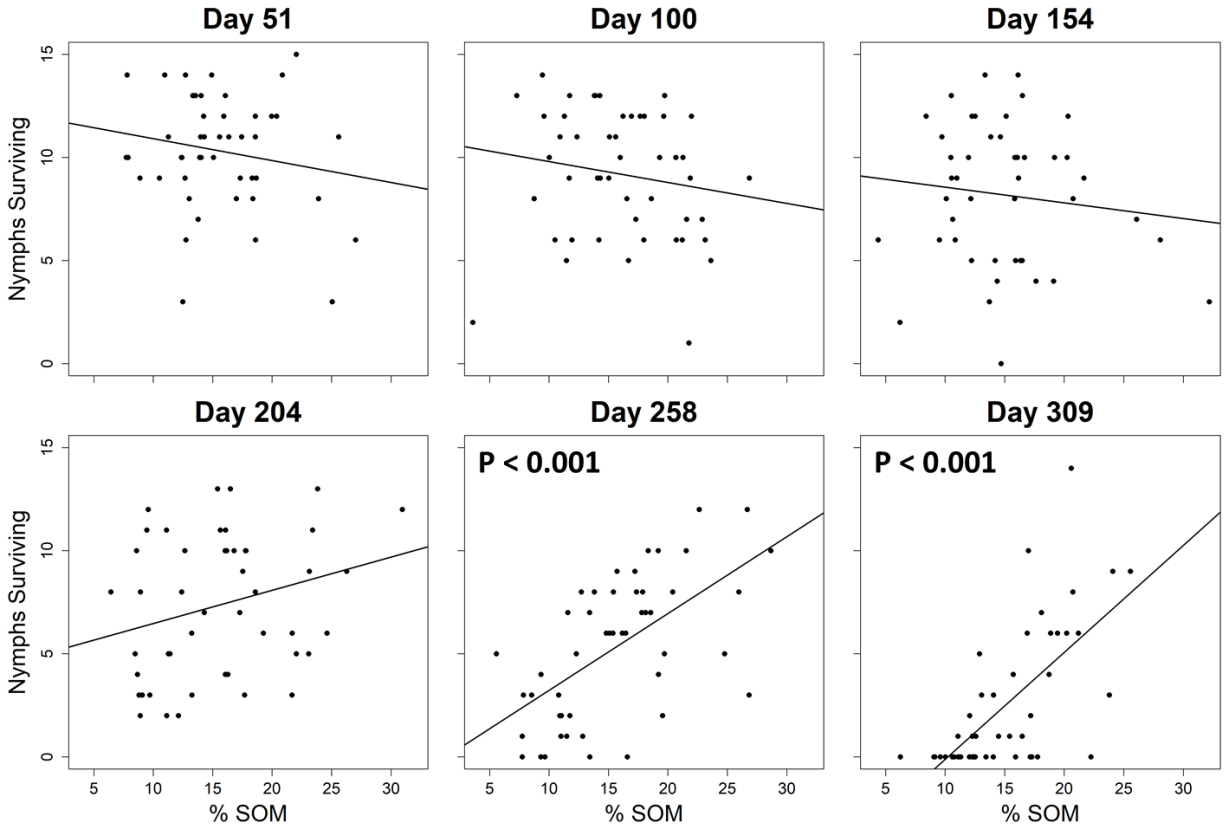

Figure 5.4: Scatterplots showing the relationship between nymphal survival and soil organic matter (% SOM) during each of the six collections. The line is the line of best fit, and no significant relationship between survival and % SOM was observed for those collection days which lack p-values in the upper left corner of their scatterplots ($P < 0.05$).

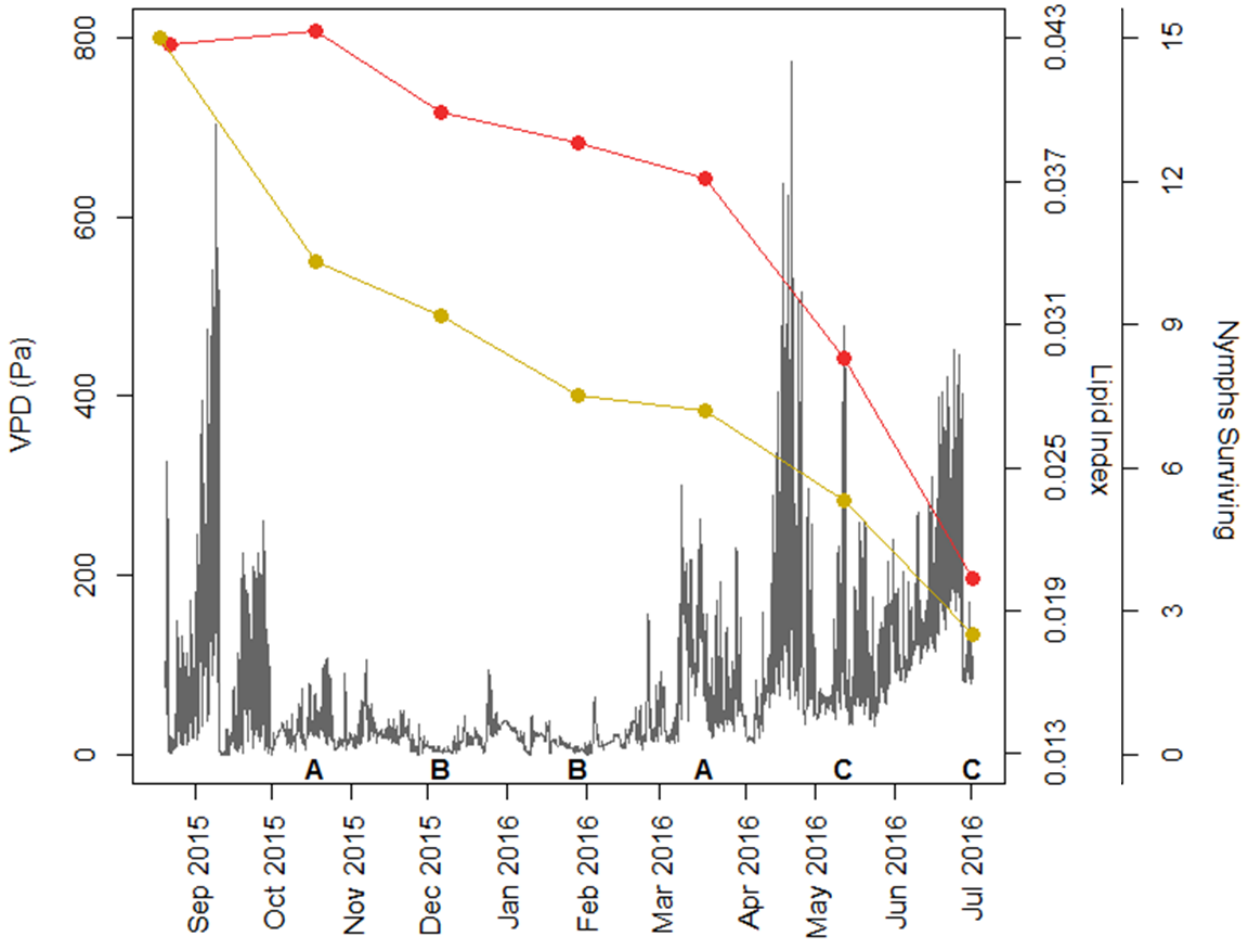

Figure 5.5: This figure shows the VPD under the leaf litter throughout the duration of the 2015 – 2016 field season. The grey line shows the average hourly VPD across all six sites, and letters represent those time periods which differ significantly ($P < 0.05$) from one another. Matching letters do not differ significantly. The red line shows the average lipid index for that collection date, whereas the gold line shows the average number of nymphs surviving in the microcosm.

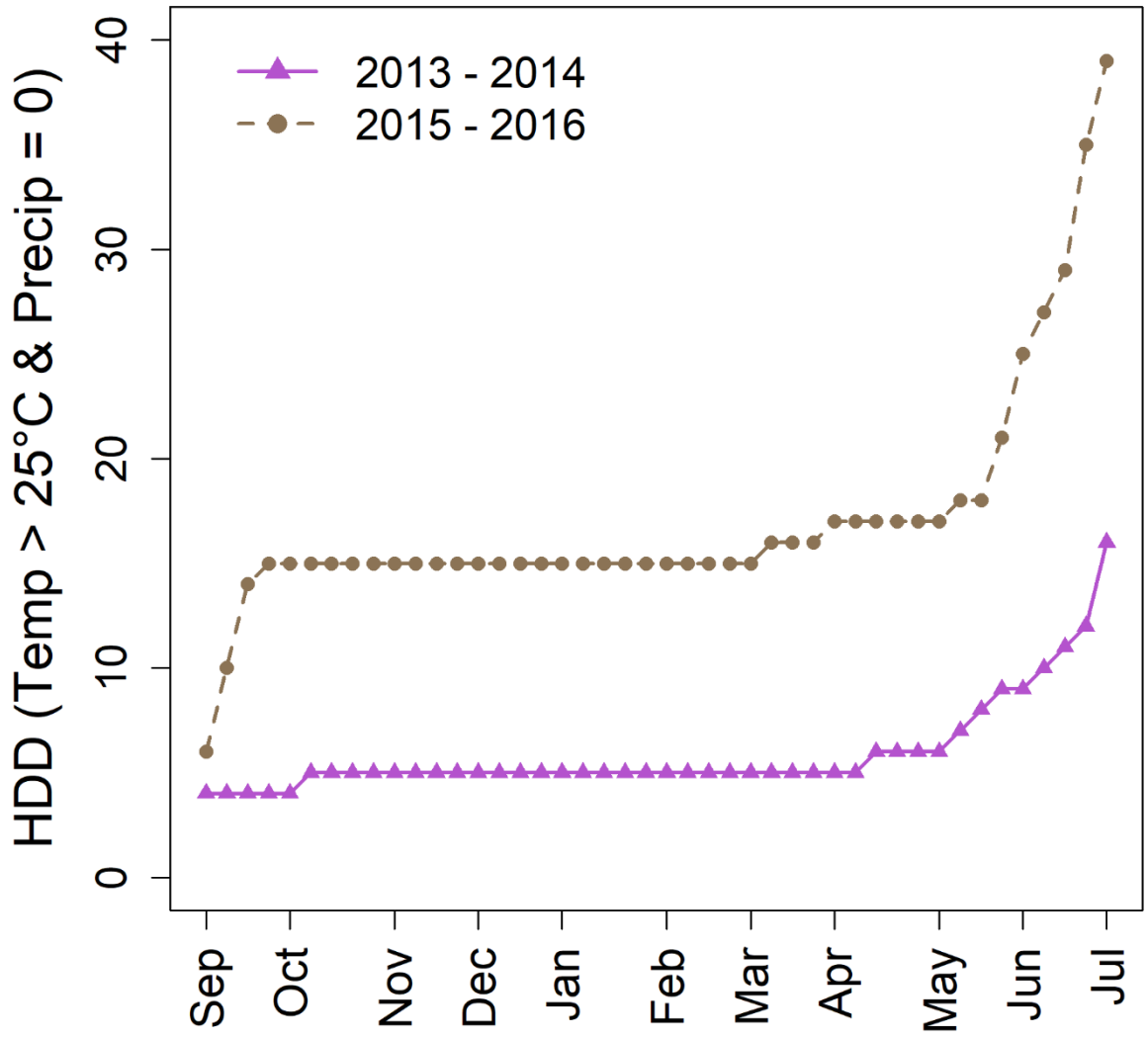

Figure 5.6: The cumulative number of hot (T < 25 °C) dry (precip = 0) days (HDD) in 2013 – 2014 (dashed line) and 2015 – 2016 (solid line). Each point on the line represents one week.

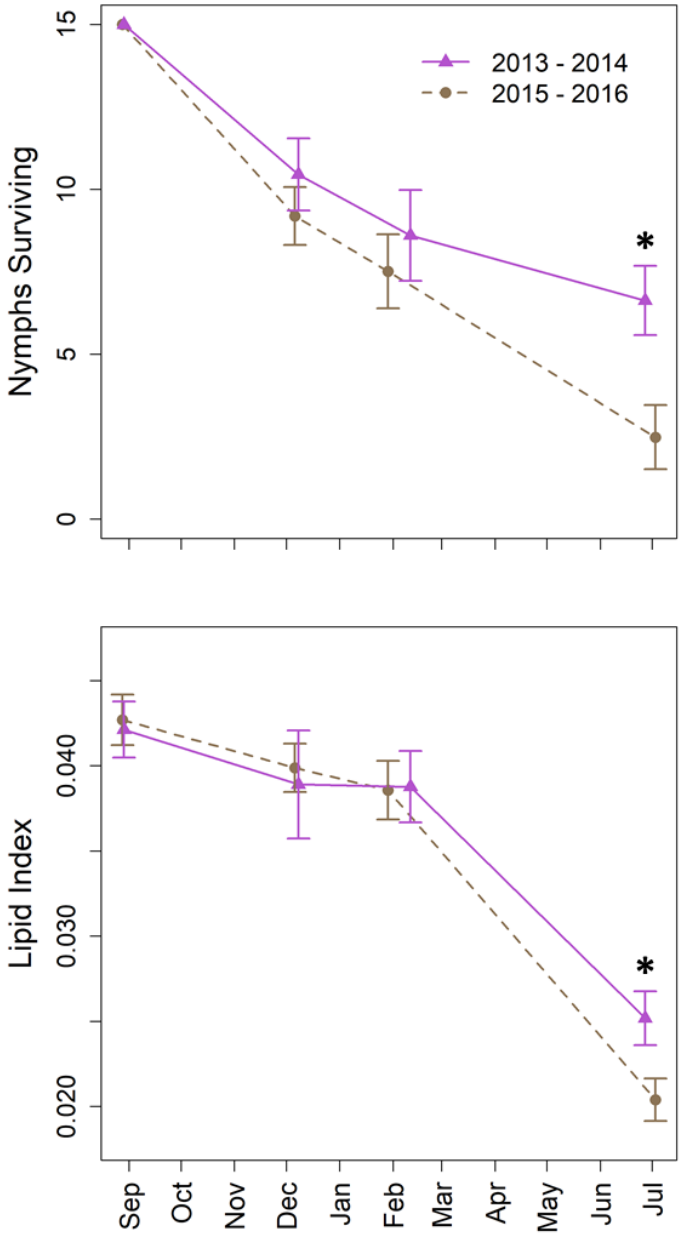

Figure 5.7: Comparison of *I. scapularis* nymphal survival (top) and physiological age (bottom) in 2013 – 2014 in Ithaca NY (solid lines) and 2015 – 2016 (dashed lines) in Dutchess County NY. Points represent the mean for that sampling period, and the error bars represent the 95% confidence interval. The * symbol represents a significant ($p < 0.01$) difference during that sampling period.

Table 5.1: The density of nymphs (DON) per 100 m^2 collected on each of the six sites in 2011 and 2012, along with the density designations used for our study.

Site	DON 2011	DON 2012	Density Category
Cary	12.7	3.8	High
Tymor	31.8	2	High
Wilcox	17.8	3.0	High
Depot	0.3	0.3	Low
Sharpe	0	1.0	Low
Taconic	1.2	2.3	Low

Table 5.2: The AIC values for the mixed effects model used to determine the relationship between tick survival in the microcosms in 2015 – 2016 with the fixed effects listed below. The 'density' parameter represents the high- vs low-density sites, 'SOM' is percent soil organic matter, and 'collection' is the date the microcosms were collected. All models, including the intercept model, include 'site' as a random effect. The ΔAIC values are compared against the intercept model.

Model	Number of Parameters	AIC	ΔAIC
Intercept	1	1638.6	0
Density	2	1638.2	-0.4
SOM	2	1592.1	-46.5
Collection	2	1505.1	-133.5
Collection + SOM	3	1449.8	-188.2
Collection + SOM + Collection:SOM	4	1410.0	-228.6

Table 5.3: The AIC values for the mixed effects model used to determine the relationship between the lipid index values of the ticks collected in 2015 – 2016 with the fixed effects listed below. Fixed effects are the same as described in table 1. All models, including the intercept model, include 'site' as a random effect. The ΔAIC values are compared against the intercept model.

Model	Number of Parameters	AIC	ΔAIC
Intercept	1	-1656.8	0
Density	2	-1638.7	18.1
SOM	2	-1643.3	13.5
Collection	2	-1797.2	-140.4

Table 5.4: The AIC values for the mixed effects model used to determine the relationship between tick survival in the microcosms with the fixed effects listed below between the two collection years. Fixed effects are the same as described in table 1, with the addition of 'year' which represents the collection year (2013 – 2014 / 2015 – 2016). All models including the intercept model include 'site' as a random effect. The ΔAIC values are compared against the intercept model.

Model	Number of Parameters	AIC	ΔAIC
Intercept	1	1317.9	0
Year	2	1316.0	-1.9
SOM	2	1288.3	-29.6
Collection	2	1231.9	-86.9
Year + SOM	3	1286.3	-31.6
Year + SOM + Collection	4	1194.3	-123.6
Year + SOM + Collection + Year:SOM	5	1197.6	-120.3
Year + SOM + Collection + Year:Collection	5	1182.0	-135.9
Year + SOM + Collection + Year:Collection + Collection:SOM	6	1168.8	-149.1
Year + SOM + Collection + Year:Collection + Collection:SOM + Year:Collection:SOM	7	1164.7	-153.9

Table 5.5: The AIC values for the mixed effects model used to determine the relationship between the lipid index values of ticks in the microcosms with the fixed effects listed below between the two collection years. Fixed effects are the same as described in tables 1 and 2. All models including the intercept model include 'site' as a random effect. The ΔAIC values are compared against the intercept model.

Model	Number of Parameters	AIC	ΔAIC
Intercept	1	-1297.8	0
SOM	2	-1296.4	1.4
Year	2	-1300.9	-3.1
Collection	2	-1494.6	-196.8
Collection + Year	3	-1495.6	-197.8
Collection + Year + Collection:Year	4	-1500.8	-203.0

Table 5.6: Results of the mixed effects model for tick survival in the microcosms in 2015 – 2016. SOM is percent soil organic matter and collection is date of retrieval for the microcosms. This model included site as a random effect.

Fixed Effects	DF	F-Value	P-Value
SOM	5, 265	27.75	< 0.001
Collection	1, 268	13.91	< 0.001
Collection:SOM	5, 265	12.10	< 0.001

Table 5.7: Results of the mixed effects model for tick survival in the microcosms between the two years (2013 – 2014 / 2015 – 2016). SOM is percent soil organic matter and collection is date of retrieval for the microcosms. This model includes three collection dates (December + February + July) and site as a random effect.

Fixed Effects	DF	F-Value	P-Value
SOM	1, 209	0.03	0.870
Collection	1, 208	14.38	< 0.001
Year	1, 208	4.77	0.038
Collection:Year	1, 208	7.50	< 0.001
Collection:SOM	1, 208	4.09	0.018
Collection:SOM:Year	1, 208	4.16	0.007

Table 5.8: Results of the mixed effects model analyzing lipid index values of ticks in the microcosms between the two years (2013 – 2014 / 2015 – 2016). SOM is percent soil organic matter and collection is date of retrieval for the microcosms. This model includes three collection dates (December + February + July) and site as a random effect.

Fixed Effects	DF	F-Value	P-Value
Collection	1, 194	173.52	< 0.001
Year	1, 194	2.53	0.113
Collection:Year	1, 194	4.57	0.012

CHAPTER 6

TICKS AS SOIL DWELLING ARTHROPODS: AN INTERSECTION BETWEEN HUMAN HEALTH AND SOIL ECOLOGY

Abstract

Ticks are widespread vectors for many important medical and veterinary diseases, and a better understanding of the factors that regulate their population dynamics is needed to reduce risk for humans, wildlife, and domestic animals. Although non-nidicolous ticks, the principal vectors for many zoonotic diseases, spend a small portion of their life cycle on vertebrate hosts, tick-host interactions play a key role in tick survival and reproduction. Hence, prediction of tick population dynamics depends in part on understanding factors affecting host populations and tick-host contact rates. These complex aspects of tick-borne disease epidemiology are comparatively well-studied, but tick survival and behavior are also affected by many off-host factors within soil environments, where non-nidicolous ticks spend the majority of their life cycle. This aspect of tick ecology has received much less attention, and our ability to better predict spatiotemporal trends in tick-borne diseases requires more knowledge of soil ecosystems and their effect on host and tick populations. Here we describe the known impact of biotic and abiotic factors within the soil ecosystem on tick survival, with particular attention to species of medical and veterinary concern. We also detail the potential for cascading effects through the soil ecosystem to influence both ticks, and the vertebrate hosts on which they feed. Additionally, we discuss the potential for the ecosystem-level impacts of tick-mediated reductions of vertebrate host populations, as well as the non-target impact of tick population management on

the soil ecosystem. Soils are complex ecosystems with enormous potential to impact the survival and behavior of ticks during their off-host periods. Hence, tick-borne disease systems present an excellent opportunity for soil ecologists and public health researchers to collaborate and improve our understanding of these medically important, and ecologically complex disease cycles.

Introduction

Ticks (Ixodida) are a diverse group of obligate ectoparasites with a nearly pan-global distribution. Currently, 896 tick species have been identified, spanning three taxonomic families (Guglielmone et al. 2014). The hard ticks (Ixodidae) are the most diverse, and this family contains most of the species that are important vectors for diseases targeting humans and domestic animals. They vector a wide variety of bacterial, protozoan, and viral pathogens (Fuente et al. 2008); one of the most prominent being the spirochete, *Borrelia burgdorferi*, the agent of Lyme disease (Schmid 1985). This agent is carried by species in the *Ixodes ricinus* species complex, which can be found throughout the northern hemisphere in temperate regions (Gubler 1998). Other common tick-borne infections with significant human health impacts include anaplasmosis, babesiosis, as well as a variety of dangerous tick-borne encephalitic viruses and rickettsial bacteria (Jongejan & Uilenberg 2004). These diseases have an enormous economic impact, with the cost of Lyme disease alone in the United States estimated at up to $1.3 billion annually (Adrion et al. 2015). Additionally, a wide variety of tick-borne pathogens target domestic animals and wildlife (Dantas-Torres et al. 2012), often causing high mortality (Uilenberg 1995). For example, *Rhipicephalus appendiculatus* is a vector for *Theileria parva*, the agent of East Coast Fever (Young & Leitch 1981), which causes distress and mortality in cattle herds, severely damaging local economies, particularly in central and eastern Africa (Kivaria 2006).

Ticks have evolved to target a wide variety of hosts and life history strategies vary among species. These strategies have important implications for how these arthropods interact with hosts, as well as their off-host environments. Species in Ixodidae feed on a wide variety of vertebrate taxa, from mammals-to-reptiles, and many are known to target humans or domestic

animals given the opportunity (Ghosh et al. 2007). Hard ticks like those in Ixodidae employ one of two general strategies, 1) nidicolous or 2) non-nidicolous. Nidicolous ticks have relatively low contact rates with people and domestic animals as they tend to stay within the protected area of a host's nest or burrow to avoid harsh climatic conditions. In contrast, except for some specialists which spend much of their life cycle on hosts, most non-nidicolous species spend long periods off-host (Sonenshine & Roe 2014). During these periods they are either searching for their next bloodmeal (questing) or in soil refugia protected from harsh climatic conditions (e.g., high or low temperature, and low humidity).

The aim of this review is to summarize the factors within the soil environment that affect the survival and behavior of non-nidicolous ticks, focusing on those species that are of medical and veterinary significance. We begin with a brief overview of tick-host interactions, followed by more detailed accounts of the direct impacts of the soil environment on tick survival and reproduction, as well as indirect impacts mediated through their hosts. We close with some exploration of the potential for ticks to affect soil food webs and recommendations for future work. We hope this contribution provides an accessible resource both for researchers and the general public.

Tick – Host Interactions

Although ticks spend a limited portion of their life cycle feeding upon them, hosts play an important role in the survival and reproductive success of non-nidicolous ticks, as for many species they represent both their food source and mating grounds (Wilson et al. 1990, Bechara et al. 1995). Tick questing behavior and host contact rates have a strong impact on the survival and molting success of ticks (Randolph 2004). Some species never leave the host, and while they can cause high degrees of mortality in livestock, these one-host species are specialists that generally

transmit pathogens to a single host species or taxa (Hoogstraal & Aeschlimann 1982). Most species that transmit zoonotic pathogens to people, wildlife, and domestic animals are multi-host species, that drop off the host after a bloodmeal and must spend a large portion of their life cycle questing or dormant in off-host environments (Needham & Teel 1991).

Host density and community composition impact both tick population dynamics and the transmission cycles of many tick-borne pathogens (LoGiudice et al. 2003, Mansfield et al. 2009, Hersh et al. 2012). For example, the population density of the American dog tick (*Dermacentor variabilis*), a widespread species in North America, is closely associated with that of one of their primary hosts in Maryland, the meadow vole (*Microtus pennsylvanicus*) (Carroll & Nichols 1986). Both *I. ricinus* and *I. scapularis* populations are also affected by the abundance of their vertebrate hosts (Ostfeld et al. 2006, Ostfeld et al. in press). The host community not only affects tick populations, but also influences the transmission cycles of many tick-borne pathogens. For example, transmission of the protozoa *Babesia microti* in Poland (Welc-Falęciak et al. 2008) and the spirochete *Borrelia burgdorferi* in the United States (Levi et al. 2016) are strongly influenced by their host communities. Host community composition also affects pathogen transmission for the tick-borne diseases of domestic animals. For example, in the louping ill virus system vectored by *I. ricinus* the relative population densities of grouse, hare, and deer can amplify or dilute the transmission cycle of the virus (Gilbert et al. 2001).

Complex ecosystem-level factors can also affect tick-host contact rates by impacting host densities (Kremen & Ostfeld 2005). One classic example that illustrates this complexity is the relationship between oak mast years and increased prevalence of *I. scapularis* infected with *B. burgdorferi*. This system operates on a two-year lag. Oak trees synchronously produce an exceptionally large crop of acorns, which dramatically increases the number of small mammals

that survive the winter. Those small mammals produce many offspring which increases tick-host contact rates and allows an above average number of larval *I. scapularis* to feed. These larvae molt and overwinter to emerge at high densities as nymphs, two years after the oak tree masting event (Ostfeld et al. 1996, Ostfeld et al. in press). Another example of a complex ecological interaction with a tick-borne infection is the indirect impact of invasive species management on the prevalence of the Lone Star Tick (*Amblyomma americanum*) infected with *Ehrlichia chaffeensis* and *E. ewingii* (Allen et al. 2010). In this case removal of Amur honeysuckle (*Lonicera maackii*) reduced the amount of time that white-tailed deer (*Odocoileus virginianus*) spent on field sites. These deer are important reservoirs for Ehrlichiosis and act as hosts for *A. americanum*. As a result, both the density of *A. americanum* and the proportion of the tick population infected with Ehrlichia were reduced when Amur honeysuckle was removed (Allan et al. 2010).

The questing behavior of ticks also affects tick-host contact rates, and reduced questing activity is common for many species under harsh environmental conditions (Randolph 2008). For example, during hot and dry conditions both *I. scapularis* and *I. ricnius* reduce questing activity to avoid desiccation, thereby reducing the probability of finding a host (Perret et al. 2000, Vail & Smith 2002). Additionally, many species at high latitudes have inactive periods during which they do not search for hosts but remain in diapause to avoid freezing damage (Belozerov et al. 2002). Randolph et al. (2002) hypothesized that protective microhabitats may shield ticks from some of these weather effects, allowing them to quest more often, but the effect of microhabitat availability on tick-host contact rates under field conditions remains unexplored. Overall, the factors affecting tick – host interactions are well-studied, but the variability of these

effects under field conditions in locations with differing off-host environments has been less commonly explored.

Direct Impact of Soil Environment on Ticks

The survival and reproductive success of non-nidicolous ticks also depends upon the environmental and biotic conditions in the soil, where they spend most of their lives. Tick mortality is often high even under ideal conditions (Troughton & Levin 2007), and for many species < 1% of ticks are expected to survive to adulthood and reproduce (Wu et al. 2013). While some species are adapted to extreme climates (Lee & Baust 1987), harsh weather conditions are a major driver for tick mortality, and these effects vary by life stage and species (Needham & Teel 1991). Ticks can behaviorally avoid these weather and climate effects by inhabiting microclimates under plants or in soil refugia (Daniel et al. 1977, Bertrand & Wilson 1996) (Fig. 6.1), but many tick species are not highly mobile (Goddard 1993) and are therefore subject to soil conditions in their drop-off location. Therefore, variability of soil properties is likely to impact tick survival, particularly during their immature life stages (Daniels & Fish 1990).

The soil environment can affect tick populations through both abiotic and biotic elements. Abiotic factors including slope aspect, hydrology, and soil texture affect the habitat quality of ticks (Guerra et al. 2002), thereby influencing both tick survival and behavior (Lindsay et al. 1998). Slope aspect affects the amount of solar radiation received at a location on the landscape. Areas that receive higher amounts of solar radiation are relatively dry, and during dry conditions many ticks reduce their questing activity (Gern et al. 2008) and mortality increases (Rodgers et al. 2007). For example, densities of *I. pacificus* are low on field sites that are exposed to high levels of solar radiation (Eisen et al. 2006a). Furthermore, the transmission of tick-borne diseases to people can also be affected, as the number of human cases of tick-borne

encephalitis has been predicted by slope aspect at small-scales in China (Li 2017). Hydrologic regime can impact tick populations, not only in relation to dry sites, but also in locations that are too wet to support tick and host communities (Eisen et al. 2006a). For example, in the humid continental climate of the North Central United States *I. scapularis* is generally associated with dry / mesic forests with sandy soils and is not found in areas inundated with water (Guerra et al. 2002).

Ticks also interact with a variety of biotic factors, including plants and soil biota, during their time in the soil environment. The presence and community composition of plants is often the most important biotic factor affecting tick densities and survival (Lauterbach et al. 2013), and the principal mechanism of vegetation effects is the provision of protection from harsh weather conditions during the long off-host period of non-nidicolous ticks. Ticks are adapted for specific habitats (Lindström & Jaenson 2003), as forest-dwelling species will not thrive in fields or grasslands (Daniel et al. 1977). Thus, reforestation has been suggested as a major driving factor for the range expansions of *I. ricinus* in Europe (Gilbert et al. 2017) and *I. scapularis* in North America (Rand et al. 2003). The effects of vegetation cover on tick populations can be remarkably consistent across species, with a study of tick densities in Mendocino County, California finding that tree community composition was a strong predictor of the presence of all seven tick species investigated, with each showing specific habitat associations (Eisen et al. 2006b).

Even within similar habitats, the community composition of plants can affect tick densities. For example, the geographic distribution of *D. andersoni* populations at local scales in British Columbia, Canada was closely tied to vegetation community composition (Schaalje & Wilkinson 1985) and *I. scapularis* densities are reduced when Japanese barberry (*Berberis*

thunbergii), and invasive shrub in North America, is removed (Williams and Ward 2010). Moreover, a recent investigation explored the effect of Japanese stiltgrass (*Microstegium vimineum*) invasion on the survival of two tick species (*A. americanum* / *D. variabilis*) in the United States. When *M. vimineum* invaded it eliminated the protective microclimate provided by native plants, resulting in increased mortality for both tick species (Civitello et al. 2008). Similarly, *I. scapularis* and *A. americanum* density increases with the depth of the litter layer (Schulze & Jordan 2005), which varies with plant litter quality and differs among plant species (Hobbie 1996). Decomposers, including earthworms, can also reduce litter layer thickness and impact tick populations by limiting soil refugia (Burtis et al. 2014). In sum, the primary direct effect of vegetation cover on tick survival is likely mediated through effects on the physical habitat and microclimate, although host communities can also be affected by vegetation (Tews et al. 2004), as will be discussed in the following section.

A number of soil-dwelling arthropod predators and entomopathogens are known to target non-nidicolous ticks under natural conditions, but research on this topic has focused on identifying potential biological control agents (Samish et al. 2004) with less attention to the effects of natural predator and pathogen communities on tick survival. A wide variety of spiders, ants, and predatory beetles will consume ticks both under laboratory and field conditions (Samish & Alexeev 2001), but the impact of natural arthropod predator communities on questing ticks remains largely unexplored. Entomopathogenic fungi also infect ticks under field conditions (Benjamin et al. 2002) and two species of fungi (*Beauveria bassiana* / *Metarhizium brunneum*) have been demonstrated to be particularly effective biological control agents that are able to target many tick species (Gindin et al. 2003). Although both fungal species have been

isolated from soils across the globe (Bidochka et al. 1998), their natural distribution in soils and interactions with tick populations are not well-characterized.

More complex interactions within and between the biotic and abiotic components of the soil environment can also affect tick populations. Dense vegetation over excessively well-drained soils may mitigate desiccation effects, and decomposer communities in different soils may reduce leaf litter depth and microhabitat availability on the soil surface under the same vegetation cover (Heneghan et al. 2007). Insect herbivores commonly impact plant communities, and grazing effects can cascade, increasing tick densities in some cases (Coyle et al. 2013). Physical soil insulators, including snow and soil organic matter are also thought to protect ticks from weather conditions (Hayes et al. 2015, Burtis & Pflueger 2017), but few experimental studies have explored these effects (Burtis et al. 2016a). Although numerous case studies have linked isolated factors within the soil environment to tick survival or density, a holistic understanding of how these factors interact to affect ticks under natural conditions is still lacking. Moreover, conclusive evidence about the mechanisms underlying observed spatial patterns of tick abundance requires experimental follow-up investigations, which are scarce (Killilea et al. 2008).

Tick-Host Interactions as Mediated by the Soil Environment

Tick populations can be indirectly impacted through factors affecting the community dynamics of their vertebrate hosts (Rosa & Pugliese 2007). Hosts are important for ticks as food sources, reproductive sites, and also as vehicles for long-distance dispersal (Ogden et al. 2008, Ruiz-Fons & Gilbert 2010). The soil environment can influence tick-host contact rates through its effects on vegetation, host populations, and tick questing behavior.

Multiple factors within the soil environment can alter the questing behavior of ticks, often by regulating questing height and microhabitat quality. Humid conditions under plants and leaf litter protect ticks during their questing periods (Bertrand & Wilson 1996), thereby increasing questing activity during dry conditions and increasing tick-host contact rates (Randolph & Storey 1999). In a microcosm study in New Zealand *A. sphenodonti* was more active when substrates were moist and shaded (Godfrey et al. 2011), and extensive research has been conducted relating the questing behavior of many tick species to the availability of humid microhabitats (Perret et al. 2000, Vail & Smith 2002). Vegetation also provides important questing habitat (Prusinski et al. 2006), particularly for species targeting large-bodied hosts (Tsunoda & Tatsuzawa 2004). A tick (*D. albipictus*) that primarily infests moose (*Alces alces*) and other large herbivores in North America relies on vegetation to climb to the torso height of their target hosts (McPherson et al. 2000). Questing height is also believed to be the mechanism by which different life stages select a particular group of hosts, with earlier life stages questing at a lower height, which brings them into contact with smaller vertebrates (Mejlon & Jaenson 1997).

Plant community composition also strongly influences the population dynamics and diversity of vertebrate hosts (Tews et al. 2004); hence, in ecosystems exhibiting bottom-up control, the soil environment, through its influence on plants (Lavelle et al. 2006), has enormous potential to impact tick populations by altering host community dynamics (Pace et al. 1999). Obviously, ticks that specialize on a single species or small group of species are closely associated with these hosts (Harrison et al. 2012), but host community composition is important for generalists as well. Host species identity affects the feeding success (Keesing et al. 2009) and molting success of ticks (Brunner et al. 2011) and hosts also play a crucial role as disease reservoirs for the transmission dynamics of tick-borne zoonoses, with reservoir competency

often varying between species (Schmidt & Ostfeld 2001). Reservoir competence is well-studied in the case of *B. burgdorferi* in the United States, where small mammals make excellent reservoirs, efficiently transmitting the pathogen between generations of ticks, whereas a high proportion of ticks feeding on alternative hosts drop off uninfected (LoGiudice et al. 2003).

Host-mediated effects can also make predicting the impact of some factors on tick population dynamics more difficult. For example, fire can cause a temporary reduction in tick density through direct injury, but the opposite effect is also possible (Horak et al. 2006). In Zimbabwe *R. appendiculatus* populations increased following a fire event as vertebrate hosts congregated to feed after fires (Minshull & Jacqueline 1982). Thus, understanding the life history of ticks is not enough, as study of their primary hosts also is required. Host community composition can also make the mechanisms driving relationships between landscape factors and tick densities difficult to identify. For example, increased *I. scapularis* densities under *B. thunbergii* (Japanese Barberry) may be driven either by improved microhabitat quality for ticks, altered host communities, or both (Williams et al. 2009). The complex and potentially contradictory effects of soil and landscape factors on tick survival and host population dynamics can limit the predictive value of trends observed in large scale surveys of tick densities (Fryxell et al. 2015).

Potential Impacts of Ticks on the Soil Food Web

As ectoparasites of vertebrates, ticks hold a unique position in the soil food web that limits their potential interactions with other soil biota. Ticks do not compete directly with other soil-dwelling arthropods for food, nor do they directly affect microbiota and plants by feeding upon them. Although ticks are eaten by soil predators and may compete with other soil-dwelling arthropods for habitat, they are not regarded as tightly linked members in soil food web models

(Scheu 2002) because they feed on vertebrates, a group of organisms not often integrated into these models. However, because many vertebrates interact strongly with primary producers, their indirect role in soil food webs can be important (Van der Putten et al. 2001). Whether through morbidity, mortality, or deterrence, changes in the abundance or behavior of herbivores can have strong top-down effects on plant communities (Augustine & McNaughton 1998, Keesing & Young 2014), which will cascade through the soil ecosystem and alter soil food web dynamics (Wardle et al. 2004).

The relationship between ticks and the soil environment is not unidirectional, as ticks may have cascading ecosystem-level effects through their influence on host populations, and active management of tick populations can affect non-target biota. Host mortality caused by tick infestation or tick-transmitted infections can dramatically reduce host populations. Domestic herd animals are especially vulnerable due to their lack of resistance and tendency to congregate in large groups (Bengis et al. 2002). These tick-borne pathogens can also spillover to wildlife communities causing mortality and creating sylvatic cycles that sustain pathogen transmission even in the absence of domestic animals (Tonetti et al. 2009). Tick infestation can also impact host survival by causing physiological stress. In North America *D. albipictus* can cause mortality in Moose (*A. alces*). When infested, moose scratch their fur off when attempting to remove ticks (Mooring & Samuel 1999), and when tick infestation is high this can increase the probability that moose will succumb to hypothermia (Samuel 2007). Hosts may also avoid areas that are highly infested with ticks; for example, when *A. americanum* densities are high, white-tailed deer (*O. virginianus*) will graze less frequently in the area (Fritzsche & Allan 2012).

Active removal of ticks through management may also affect the soil ecosystem. Ultimately, the removal of ticks from the soil food web has limited potential to directly affect its

overall stability because of the high degree of functional redundancy in most soil food webs (Setälä et al. 2005), as well as the fact that few known predators or pathogens rely on ticks as a primary food source. Although ticks are targeted by some arthropod predators and entomopathogens, these species tend to be generalists that will feed on a variety of alternative prey (Samish et al. 2004). Furthermore, ticks are relatively small, so it is unlikely that they comprise a substantial meal for many arthropod predators, which generally rely on larger prey items for survival (Griffiths 1980). One of the few tick-targeting specialists is the parasitic wasp *Ixodiphagus hookeri*, which will target most tick species and relies on them for reproduction (Stafford et al. 2003). This wasp can be abundant when tick densities are high, but their role in soil food webs is unknown. Overall, the removal of ticks and the few specialist species reliant upon them is unlikely to cause significant destabilization of soil food webs.

Destabilization of food webs may result from the non-target effects of tick management on soil biota (Peter et al. 2005). The most commonly employed tick control methods involve spraying infested areas with acaricides or isolates of entomopathogenic fungi (Piesman and Eisen 2008). The acaricides are not specifically targeted to ticks and will impact the natural communities of soil-dwelling arthropods (Sánchez-Bayo 2012), many of which hold important positions across trophic levels in soil food webs (Scheu & Falca 2000). Hence, the broad scale application of acaricides has the potential to destabilize soil food webs, which will potentially cascade to negatively affect nutrient cycling and plants (Wardle et al. 2004). Entomopathogenic fungi, commonly *B. bassiana* and *M. brunneum*, appear to be a less harmful method for tick management (Kirkland et al. 2004). The non-target effects of these pathogens are less severe than those of acaricides (Fischhoff et al. 2017), but some taxa are still negatively impacted by the application of these biological control agents (Saito & Brownbridge 2016).

An alternative method to the broad-scale spraying of pesticides is their direct application to vertebrate hosts. This method can effectively control the populations of several important tick-borne disease vectors (Leemon et al. 2008, Williams et al. 2018) and should limit non-target effects on soil-dwelling organisms (Fig. 6.2), but direct applications for wildlife host populations may prove challenging for practical reasons. Pesticide resistance can also impact the efficacy of these methods over time (Wharton & Roulston 1970), and other methods have been explored that focus on the management of host populations (Van Buskirk & Ostfeld 1995) or vegetation (Williams et al. 2009). These management techniques also have the potential for non-target impacts on the soil ecosystem, but could be combined with other management methods to limit non-target effects. As tick-targeting management activities intensify (Keesing & Ostfeld 2018), the measurement of these non-target effects, and research focusing on integrated pest management solutions for ticks will become increasingly important.

Conclusions and Future Directions

Tick-host interactions and their impact on the transmission of tick-borne diseases have been broadly studied, but there are still major knowledge gaps regarding the factors that affect ticks during their off-host periods. This is partially due to the difficulty of measuring tick mortality when they are not actively questing (Dobson et al 2014). Furthermore, tick-dragging, the most common census method, often underestimates tick populations (Daniels et al. 2000). This method also relies on ticks to be actively seeking hosts, so factors affecting questing behavior (e.g. low humidity) (Vail & Smith 2002) and variation in host densities (Brunner & Ostfeld 2008) may interfere with collection efficiency. These effects are difficult to account for, therefore alternative methods, including microcosm studies and body burden measurements, must be employed in conjunction with tick-dragging methods to account for these confounding

factors. Additionally, experimental manipulation of field conditions is necessary to understand the mechanisms driving the trends observed in large-scale correlative studies. Combining data from common surveillance methods and field microcosm studies can allow researchers to isolate and identify the mechanisms by which site-based factors impact ticks (Table 6.1). This approach has been employed to predict the range expansion of *I. scapularis* in Canada (Leighton et al. 2012), and the resulting models have strong predictive power (Clow et al. 2018). Ultimately, we must improve our ability not only to predict range expansion, but also interannual variation and the fine scale distribution of ticks in areas where tick-borne diseases have a long endemic history (Burtis et al. 2016b).

Moving forward, integrating the effect of landscape factors into conceptual models of tick life cycles will be important as researchers attempt to predict the geographic distribution of tick-borne disease vectors at fine- and coarse-scales across the globe. We should also focus on tick species besides those which are most commonly studied (Pfäffle et al. 2013), particularly considering the potential for other endophilic tick species to vector pathogens in their zoonotic cycles (Perez et al. 2017). Because of the large proportion of their life cycle that many important tick-borne disease vectors spend in the soil, both the biotic and abiotic components of the soil ecosystem have the potential to dramatically impact tick populations. As climate and habitat suitability change over time, ticks may either experience environmental stress or improvements in habitat quality. Many components within the soil environment have the potential to mitigate or enhance the effect of these stressors. Future collaborations between soil scientists and vector biologists will improve our ability to understand and predict the population dynamics of these important and globally distributed disease vectors.

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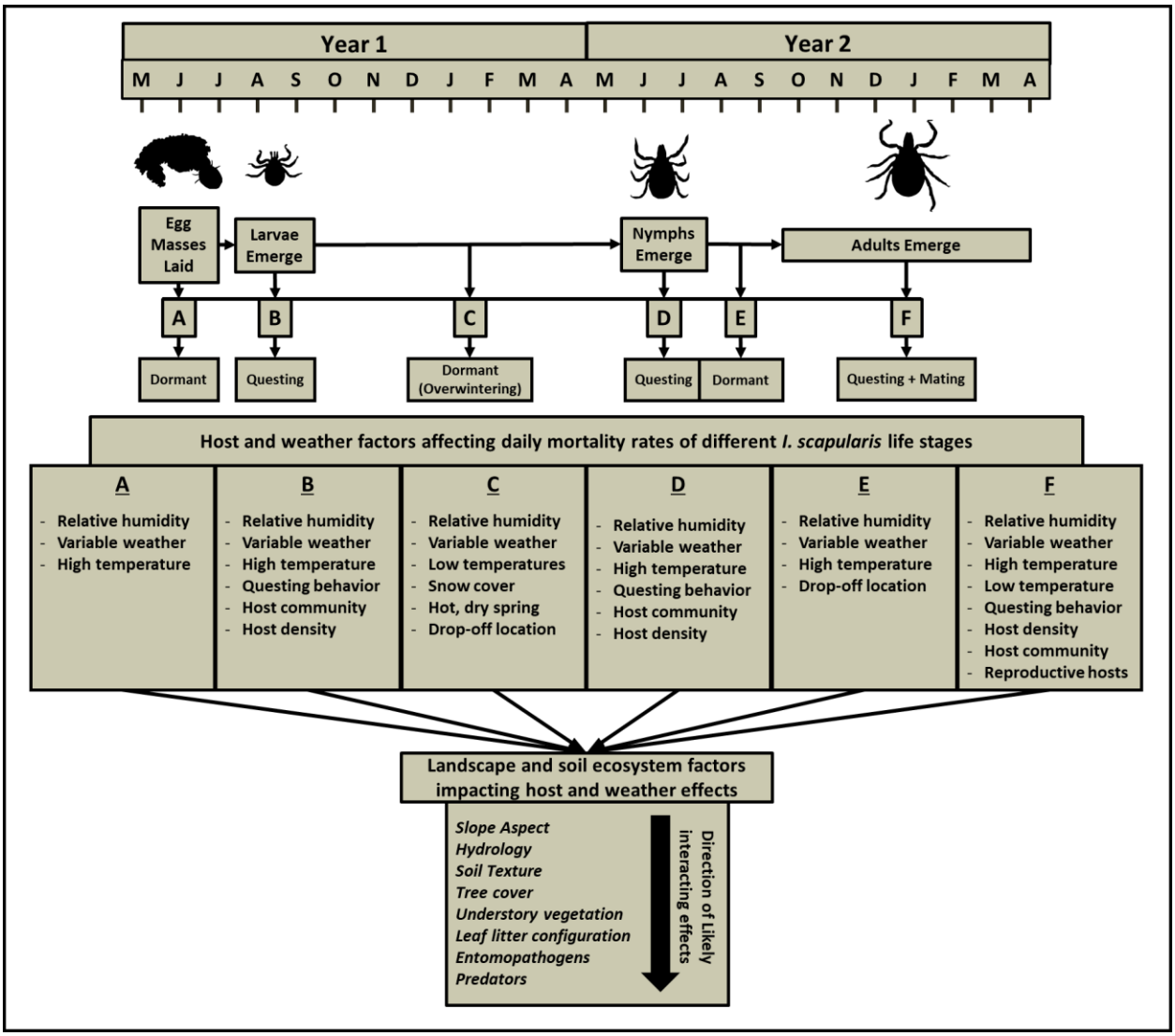

Figure 6.1: Diagram illustrating the different factors that affect the daily mortality rates of *I. scapularis* throughout its two-year life cycle during both their questing and dormant periods. The letters (A – F) correspond to the portion of the tick life cycle during which the listed factors affect tick survival. The bottom box contains landscape and soil ecosystem factors that have the potential to alter host communities and weather effects on *I. scapularis*. The downward arrow indicates the likely direction of interacting effects between landscape and soil ecosystem factors.

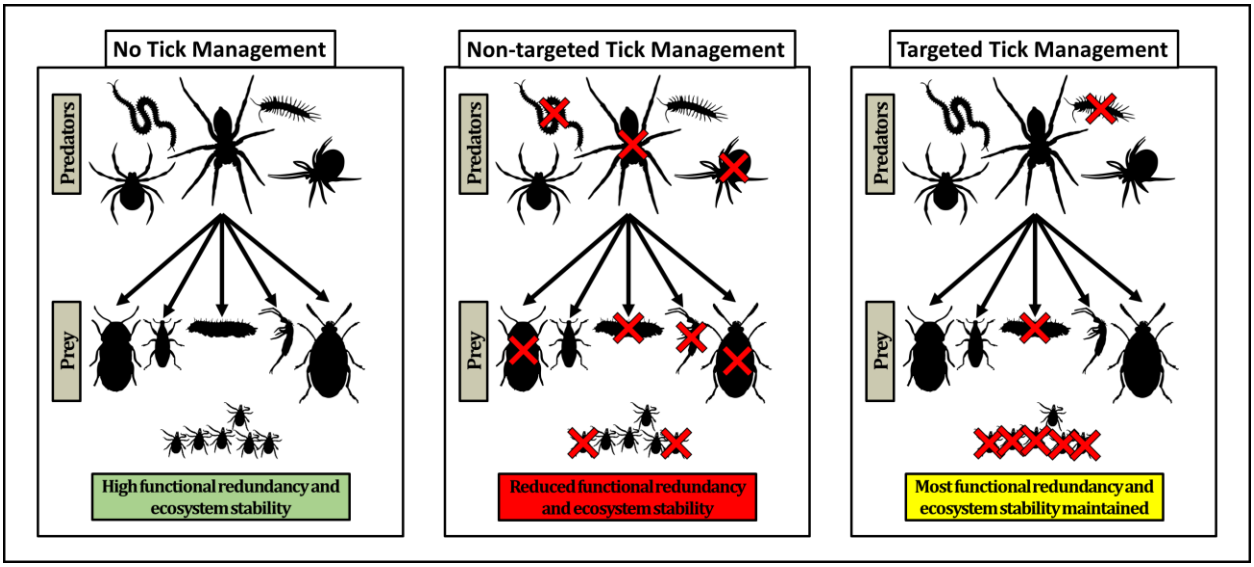

Figure 6.2: The potential effect of non-targeted and targeted methods of tick management on the communities of soil-dwelling arthropods. Due to the high functional redundancy of the soil ecosystem the removal of ticks as a prey item is unlikely to impact the stability of the soil food web. When no tick management is applied, soil ecosystems have a high degree of functional redundancy, but when non-targeted tick management (e.g. ground spraying) is employed many species may be eliminated due to non-target effects. When a targeted management technique is used (e.g. acaricide application to animals) these secondary effects should be reduced.

Table 6.1: Research questions along with relevant observations and possible future investigations focusing on the impact of the soil ecosystem on tick survival and density, with special reference to the understudied components of tick-borne disease systems.

Research Questions	Relevant Observations	Future Investigations
Can factors within the soil ecosystem explain spatial variation in tick densities at fine scales?	- Tick densities are spatially heterogenous at fine scales	- Improve habitat suitability models by including landscape and soil factors which affect tick survival and density
	- Vegetative cover can affect tick survival and host communities	- Investigate whether vegetation community composition affects tick survival and host community composition
	- Tick-targeting predators and entomopathogens can reduce tick survival under field conditions	- Determine the natural distribution of tick-targeting predators and entomopathogens
Can interactions between soil ecosystem parameters and weather conditions mitigate the impact of climate change on tick survival?	- Soil organic matter and leaf litter may protect ticks from desiccation	- Manipulation of soil insulation under controlled environmental conditions to explore effects on tick survival
	- Snow cover insulates the soil in the winter	- Removal of snow cover to detect insulation effects on overwinter tick survival
	- Temperature and relative humidity differ significantly between the air and soil environment	- Use of soil temperature and relative humidity estimates when calculating tick habitat suitability
Are trends in large observational data sets of tick densities a result of direct survival and / or host-mediated effects?	- Many relationships between tick density and landscape factors have been observed in large observational data sets	- Field manipulations to explore underlying mechanisms driving the correlations present in survey data
	- Landscape factors can affect tick survival either directly or indirectly through hosts	- Use of multiple tick data collection metrics to identify mechanisms affecting densities of questing ticks
What are the non-target effects of different tick management methods?	- Pesticides are often associated with dramatic non-target effects that can affect soil food webs	- Inclusion of ecosystem-level impacts in the evaluation of novel tick control methods